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THE INFLUENCE OF CERTAIN TREATMENTS UPON SOLANINE SYNTHESIS IN POTATOES

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April, 1953

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The Influence of Certain Treatments upon Solanine Synthesis in Potatoes

(Abstract)

A series of field, greenhouse, storage and irradiation experiments on potatoes were undertaken in order to investigate many possible factors that might be associated with abnormal solanine synthesis in potato tubers.

In the field plot, three potato varieties - Netted Gem, Carter's Early Favorite and Katahdin - were the subject of investigation. In storage experiments Netted Gem and Katahdin, and in the remaining trials Netted Gem potatoes only, were involved.

The evaluation of results was based on chemical analysis of tubers for solanine content according to the procedure described by Dabbs. All determinations were expressed in milligrams per 100 gm. of whole-tuber tissue.

(1) Various experimental treatments were applied to field-grown plants at the end of the vegetative period. None of the treatments applied appeared to have any appreciable effect on the amount of solanine present in freshly

harvested tubers. Netted Gem seed pieces with abnormally high solanine content, planted in the field, produced tubers which had a normal content at the end of the season. A very rapid and significant increase of solanine concentration resulted in freshly harvested tubers when they were exposed to solar radiation for intervals as short as six hours. Thus, it appears that the commercial practice of spreading tubers to dry on the soil surface is a dangerous one, and will result (at least in some predisposed varieties) in the development of a high degree of bitterness.

(2) Relatively high solanine content was found in greenhouse-grown tubers of Netted Gem variety, when compared to those harvested from the field plot. The high concentrations were considered to be due primarily to abnormal growing conditions (inadequate growing space and relatively high night temperatures, resulting in abnormally small tubers), rather than to the experimental treatments imposed. As the addition of phosphorus resulted in a 25 percent decrease of solanine content in spite of the abnormal growth of tubers, it appears that an upset of the balance of essential nutrients may influence solanine synthesis.

(3) A study of various storage conditions over a period of six weeks disclosed that low temperature is a primary factor contributing to excessive solanine formation

in Netted Gem, whereas Katahdin shows relatively little response. Increases of solanine at weekly intervals, when tubers were kept at low temperatures in the dark, was slow and gradual; exposure to continuous weak illumination resulted in an abrupt and rapid increase after one week, and eventually dangerous and allegedly toxic levels were reached. High temperatures appear to have a retarding effect as low solanine content was found repeatedly in both varieties whether they were kept in the dark or exposed to continuous illumination. Possibly high temperatures may bring about an actual decrease of solanine content.

(4) In the irradiation experiments, two components of radiant energy spectrum, just below and above the zone of visible light (infra-red at 13,000 Å, and ultra-violet at 3654 Å), were utilized to determine their effect upon solanine synthesis.

(a) Active tuber material after eight to nine months of cold storage has shown a considerable variability of solanine, which was attributed to resumed metabolic activity rather than to the irradiation treatments. The solanine content under infra-red illumination was found to be above, and that of ultra-violet exposures more or less below, that of non-irradiated tubers.

(b) Continuous exposures of freshly harvested dormant tuber material to infra-red light induced a very rapid and significant increase of the glyco-alkaloid content up to

dangerous levels, and an additional increase was noted when treated tubers were cold-stored for two months following the illumination. Infra-red treated tubers developed heavy chlorophyll in outer cell tissue, and when these were cold-stored, anthocyanin pigmentation also occurred. In a relatively small number of samples, a peculiar response to ultra-violet light was noted, namely a gradual increase at temperatures above 14° C., while below 10° C. a rather abrupt decrease of solanine content was noted. No chlorophyll formation resulted on exposure to ultra-violet light.

Thus from solar radiation, low temperature and long wave-length effects, it can be safely concluded that the dormant period of the tubers is the most critical stage at which, at least in the Netted Gem variety, very rapid synthesis of solanine up to dangerous and allegedly toxic levels may take place.

In the light of recent achievements of biological sciences, a hypothesis has been advanced, attempting to elucidate the predisposition of some potato varieties to form readily abnormally high solanine concentrations.

UNIVERSITY OF ALBERTA

THE INFLUENCE OF CERTAIN TREATMENTS UPON SOLANINE SYNTHESIS IN POTATOES

A THESIS
submitted to the School of Graduate Studies
in partial fulfilment of the requirements for the degree
of Master of Science

Faculty of Agriculture
Department of Plant Science

by

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EDMONTON, ALBERTA
April, 1953

Thesis
1953
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INTRODUCTION

The peculiar occurrence of bitterness in tubers of Netted Gem potato variety, which does not seem to be confined to any particular growing area, time of the year or storage conditions, has become a subject for extensive study at the University of Alberta.

The fact that potato bitterness is reported each year from various parts of the Province in the Netted Gem and other varieties is of considerable economic and commercial concern, as it is feared that it may result in serious acreage and sales reduction.

With regard to potato tuber quality, a detailed knowledge of conditions and factors responsible for unpalatable and often very acrid flavor of potatoes is necessitated in order to establish any possible remedial or preventive measures.

Numerous investigators have traced the bitter component to be the glyco-alkaloid "solanine," which occasionally occurs in potatoes in abnormally high and toxic amounts. In spite of the work that has been carried out in Europe and the United States during the past half-century, little of positive nature is known regarding the factors responsible for increased solanine synthesis and variations in quantity as demonstrated by various analytical techniques.

A new fundamental basis for solanine investigations at this institution was established by the shortened analytical procedure of Dabbs (14), which compared to tedious and time-consuming older methods allows numerous and accurate analyses in relatively short time. The effect of various cultural practices upon tuber yield and solanine content was investigated at the same time by Hutchinson (25).

The study to be reported here, which is a continuation of previously mentioned worker's research (25), was concerned with solanine synthesis as affected by certain field treatments, various greenhouse and storage conditions, and specific irradiations applied to active and dormant potato tubers.

LITERATURE REVIEW

The potato plant, Solanum tuberosum, which is of enormous economic importance for the world food production, has given its name to the large plant family of Solanaceae which is known to produce a variety of alkaloids.

A large group of tropane alkaloids is synthesized by such solanaceous species as Atropa, Datura, Duboisia, Hyoscyamus, Mandragora, Scopolia and Solandra, in which atropine, belladonine, hyoscine, tropacocaine and other alkaloids

are produced (21). The steroidal alkaloids of the Solanum species include solanine and solanidine (S. tuberosum), solanine-s and solanidine-s (S. sodomeum), solauricine (S. auriculatum) and solangustine (S. angustifolium), the first two of which are of primary interest (21).

As early as 1820 Desfosses, as reviewed by Conner (12) and Henry (21), isolated from Solanum nigrum (Black Nightshade) a basic-reacting compound named solanine, which was six years later found to be present in cultivated potato plants. Solanine is actually a glycoside, the aglycone portion of which is uniquely formed by a true alkaloid known as solanidine (11, 12, 21, 40). A third related compound was isolated by Soltys and Wallenfels and named solanthrene, which is suggested to arise as a dehydration product of solanine (40).

Of the vast number of nitrogenous bases, known as alkaloids, which occur irregularly scattered throughout many species of the plant kingdom, only two basic groups, aside from purines and pyrimidines, have been studies extensively from the viewpoint of plant biochemistry and physiology (8).

Nicotine, the principal alkaloid of the tobacco plant, was extensively studied because of its economic importance, and the site and probable pathways of its synthesis have been relatively well demonstrated (8). Also, the forma-

tion of tropane alkaloids is relatively well understood and elucidated (26, 27). There is, however, little positive information on factors and possible intermediary metabolic reactions involved in biogenesis of solanine in the potato plant. This fact is primarily due to the very complex chemical nature of the compound, and to serious difficulties arising during attempts for quantitative estimation (39).

Alkaloids, in general, are very complex nitrogen-containing compounds of basic nature, as indicated by their properties and name (alkali-like), since they form water-soluble salts with acids (21). All these compounds have a remarkable physiological action within animal bodies and have become of primary interest for animal biochemists and pharmacologists as a source of many effective and useful drugs (17).

Insofar as the plant biochemistry is concerned, alkaloids as yet have no definitely known function assessed. Bonner expresses his doubt whether they can play any general biochemical role in view of their limited and irregular distribution in the plant world (8).

Alkaloids have been variously dismissed as excretion or sometimes nitrogen reserve products of singular ineffectiveness, or poorly guessed as detoxication mechanisms (26). More probably they may represent by-products of active

metabolism as secondary products irreversibly formed for various reasons from accumulated essential metabolites (8).

Such examples of secondary by-products are known to be formed in mutant strains of Neurospora (bread mold), where a genetic block leads to accumulation of metabolites, which are then secondarily transformed (4, 23). Also, it should be borne in mind that any possible functions of these substances in organs of deposition might be quite different from that in situ of their synthesis (8).

Nevertheless, the fact that some alkaloids generally appear at the very early stage of plant development, being synthesized only in actively growing meristematic tissues, and frequently disappearing towards the end of the vegetative period (27), seems to point towards some reason for their existence.

It was only around the turn of the century that interest has been aroused as to the possibility of environment factors, cultivation and storage conditions being responsible for the occasionally reported abnormal accumulation of solanine in potato tubers (34). When this alkaloid is present in relatively large amounts in potatoes, a pungent bitter flavor is noticeable, and frequently, if such potatoes are consumed, a danger of poisoning may become acute. Von Morgenstern was first to correlate high solanine content with

bitterness in tubers of some European potato varieties (34), and his findings were confirmed when, in alleged cases of poisoning reported from many countries in certain seasons, the responsible toxic substance was identified as solanine, originating in bitter potatoes (20).

The most serious difficulties of solanine investigators arise from arduous and slow quantitative analytical methods calling for extremely careful technique (39). Therefore, it is not surprising to find frequently conflicting, inconclusive and non-comparable analytical data, as demonstrated by various isolation and determination methods employed (14). Although the tedious gravimetric procedures of earlier workers (7) were considerably improved by Conner's hydrolytic method of determination (12), and Pfankuch's colorimetric estimation of solanine (37), the extraction methods remained a slow and arduous procedure.

These methods were critically studied and modified by Rooke and co-workers (39), and it was made possible by the simultaneous use of colorimetric and hydrolytic methods to determine solanine and solanidine separately. In a survey of solanine content of American potato varieties, Wolf and Duggar introduced other modifications of extraction procedures (46), and further improvements were achieved recently in the new shortened Dabbs' extraction method (14).

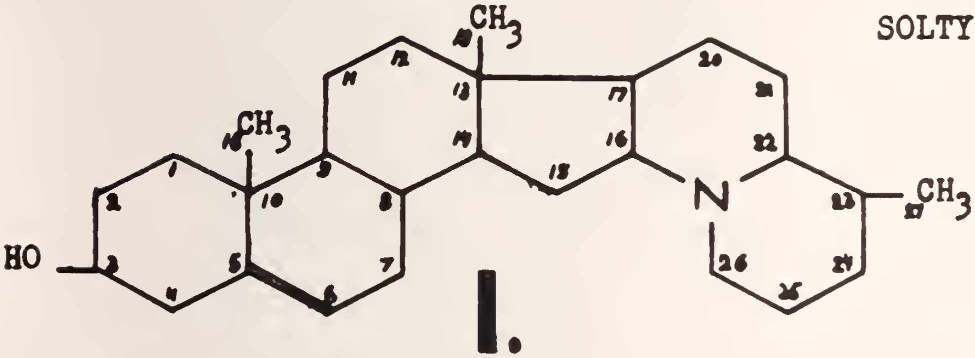
The fact that almost every worker in this field modified the existing methods of extraction to suit his own investigation accounts for much of the lack of uniformity in results and variations of alkaloidal content as determined by various techniques. The known variation in solanine content within and between potato varieties is, of course, another contributing factor.

The chemical structure and properties of solanine and related compounds have been studied by numerous workers. However, as yet there is no general agreement as to the final structure of solanine (11, 21, 40).

Soltys and Wallenfels have demonstrated the presence of a steroidal nucleus with a single double-bond (C_5 - C_6), the nitrogen portion of the molecule being attached in the form of a lupinane (40). Clemo et al favor the structure in which a methyl pyrollizidine is attached through a single C_{17} carbon atom (11). The most probable structure has been presented by Swiss workers Prelog and Spitzvogel, as reviewed by Henry (21). All three of the suggested formulas of solanidine are presented in Figure 1. To this aglycone portion of solanine molecule, three sugars are attached in C_3 position in order as listed - glucose, galactose, rhamnose.

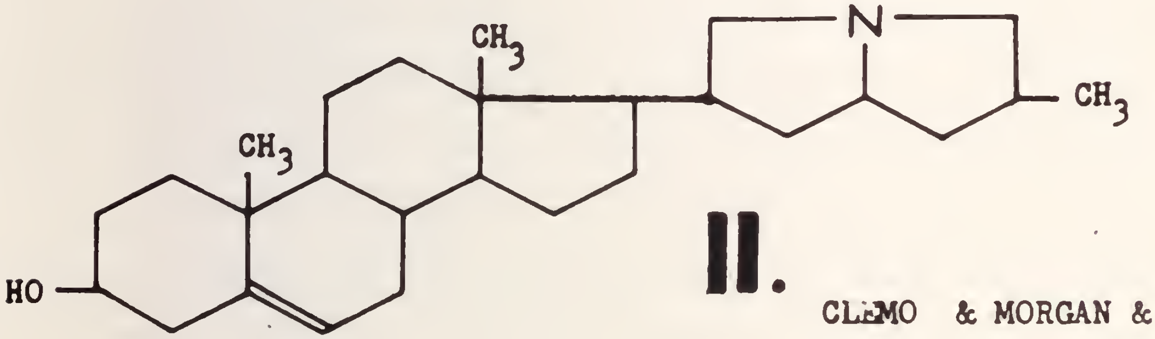
The first important study concerned with the nature of solanine synthesis in potato plants under varying

Fig. 1. Structural formulae of solanidine
as postulated by different groups of workers
and reviewed by HENRY (21).



I.

SOLTYS & WALLENFELS



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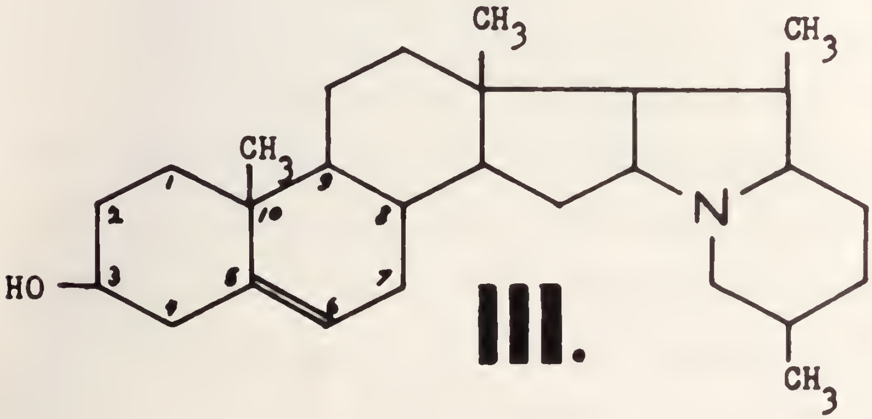
CLEMO & MORGAN & RAPER



III.

PRELOG & SPITZVOGEL

environmental conditions was reported by von Morgenstern in 1907 (34). He first associated high solanine content with occasional bitter and acrid flavor of some European varieties. The average solanine content was found to be about 0.0125 percent of fresh weight in 18 varieties, the yellow tubers having generally a lower content than tubers of blue or red varieties. Greening of tubers resulting from exposure to daylight, and nitrogen applications, are reported to increase, while potassium and ample soil moisture decrease, solanine content.

Since this first publication, the problem of potato bitterness and factors responsible for excessive solanine synthesis has been explored from various viewpoints in Europe, mostly in Germany and Great Britain. The work of Rooke, Lampitt and co-workers, has fairly well established standard solanine determination methods (39), and has shown fairly well the distribution of the complex glyco-alkaloid throughout the entire potato plant (28).

Relatively little attention was given to the alkaloidal content of American potatoes until a detailed survey was presented by Wolf and Duggar (46). Conner's investigation was carried out on a single variety only, and was primarily concerned with the effectiveness of various light qualities upon alkaloidal synthesis (12). The experiments of Mayfield et al aimed to determine the C-vitamin content of potatoes as affected by winter storage, and also reported some results of

solanine analyses. Their results are questionable because they claimed a very high solanine content of 0.050 percent (which is far above toxic levels, even in non-bitter Bliss Triumph potatoes) (32).

An extensive research program on potato bitterness is currently being carried out at the University of Alberta with relation to a study of factors affecting potato tuber quality (14, 24). The study was begun by establishment of the shortened analytical procedure by Dabbs (14), and was continued by Hutchinson in attempts to determine the effect of cultural practices upon tuber yield and solanine content (25). Hutchinson has presented an extensive review of the problem and the work carried out to date. For this reason, the opinion of the majority of workers will be summarized in order to avoid unnecessary repetition. More detailed attention will be paid to solanine synthesis aspects as related to experimental work reported here, and a few viewpoints will be discussed in the light of latest biochemical achievements.

A brief presentation of concise aspects of the solanine and potato bitterness problem as established and favored by a majority of workers in this field is given in the following paragraphs.

I

There is little doubt that solanine, or its alkaloidal component solanidine, is responsible for the bitter and acrid flavor of potatoes if present in larger-than-normal amounts (7, 30, 34, 39, 45). The upper safety limit for food purposes as recommended by Bomer and Mattis is generally agreed upon (7), and consumption of potatoes with solanine content exceeding 0.020 percent of fresh weight may bring about toxic effects, serious illness and eventually death (20). Hansen reports, however, that alleged poisoning with bitter potatoes cannot be attributed to solanine but to some form of bacterial toxin, and it would be desirable to obtain more positive evidence as to this fact (19). The observations of Mayfield et al may support this idea, as they found distinct bitterness in yellow-fleshed Netted Gems and no such flavor in the Bliss Triumph potatoes in spite of the fact that solanine content of both was claimed to be on the same level (32). Nevertheless, with regard to abnormally high concentrations of 0.050% and more (determined by a doubtfully reliable gravimetric method), these results are to be seriously questioned.

II

Solanine alkaloids are irregularly distributed throughout the whole potato plant, the free base also occurring in some varieties at certain developmental stages (11, 34). The highest alkaloid concentration is found in flowers, sprouts

and leaves. Within the tuber itself, which has generally a low content, solanine occurs mostly in the eyes, sprouts and in a thin layer of parenchymatous tissue just below the skin (28). Thiele and Fischer stated that most of the alkaloid is present in a 10-cell layer of the cortical tuber tissue (16). The general opinion, also supported by evidences of distribution of other alkaloids (26, 27), maintains that the highest concentration occurs in tissues where metabolic activity is the greatest (eyes, sprouts, active meristems, shoot tips), whereas a degradation of solanine apparently sets in at the end of the season in senescent tissues (26, 28, 34).

III

The normal solanine content in potato plants and tubers is not a constant value, and its fluctuations seem to depend mainly upon three factors: (a) variety, (b) evolutionary stage, and (c) external environment.

(a) Varietal differences in solanine content are at least to some extent accounted for by inherited genetic constitution, since it has been found feasible to increase alkaloidal content, in tobacco for instance, by selection and breeding (13). The predisposition to synthesize higher-than-normal amounts of solanine must be related to some genetic factor, as it is often found that some varieties synthesize

solanine much more readily than others (24). Presumably, it is not the amount, but the potential ability to synthesize solanine which is inherited, as it was found that solanine-rich potatoes used as seed usually produce tubers of normal solanine content (7, 45).

Von Morgenstern reported as normal a concentration of 12.5 mg. percent of solanine (34), Bomer and Mattis 2 to 8 mg. percent (7), Pfankuch 3 to 7 mg. percent (37), and Lampitt et al 7.5 mg. percent (28), all for European varieties; while Wolf and Duggar found the solanine content of American potato varieties to range from 1.8 to 13 mg. percent of fresh tuber weight (46).

The range of normal solanine contents as listed above seems to have a rather relative than absolute value, not so much because of the variability resulting from imperfect determination methods noted earlier (14, 39), but also because of the almost certain interaction of hereditary and environmental factors.

(b) The stage of plant and tuber growth is the second factor determining solanine content of each particular portion, probably by virtue of the type of intermediary metabolic processes taking place. Immature small tubers exhibit a rather high solanine content, while mature and large tubers generally show much lower concentration (7, 34, 40). Accord-

ing to Wolf and Duggar, tuber size definitely has to be taken into consideration if comparable results are to be obtained (46). According to Bomer and Mattis, young and mature tubers that are exposed to conditions favoring solanine synthesis are usually more likely to develop higher concentrations of the compound than are older tubers with a long storage history (7).

Investigation of changes in solanine content throughout the vegetative period have revealed that the concentrations in various parts of the plant are highest at the beginning and decrease towards the end of the season. On the other hand, there is still an overall increase of solanine per plant, as shown in Figures 2 and 3. These graphs were adapted from Wolf's and Duggar's report (46), because the variety in question is of primary interest in this work. Netted Gem is a synonym for Russet Burbank variety.

(c) Solanine content as determined by variety and evolutionary stage is considerably affected by external environment, which probably in most instances is responsible for abnormal production of this alkaloid. Environmental factors such as growth conditions defined by soil, climate and cultural practices (2, 3, 11, 34, 36, 46), different sources of light, solar radiant energy, spectrum components (7, 12, 28, 30), and various storage conditions (1, 7, 22, 24, 28,

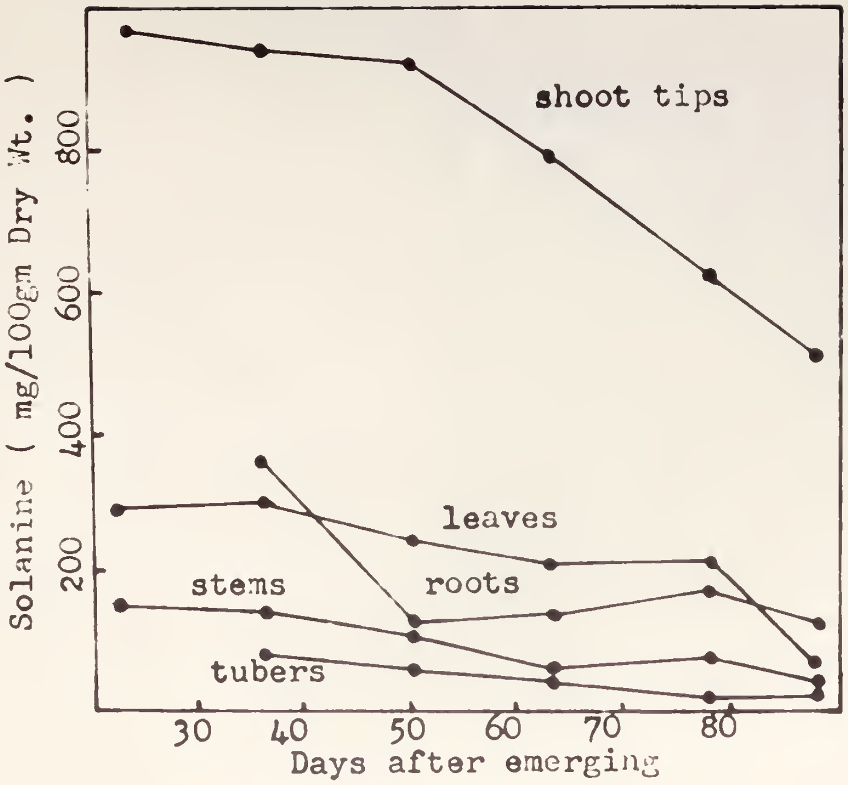


Fig. 2. Solanine content, dry weight basis, of various parts of the developing potato plant (Russet Burbank); according to Wolf and Duggar (46).

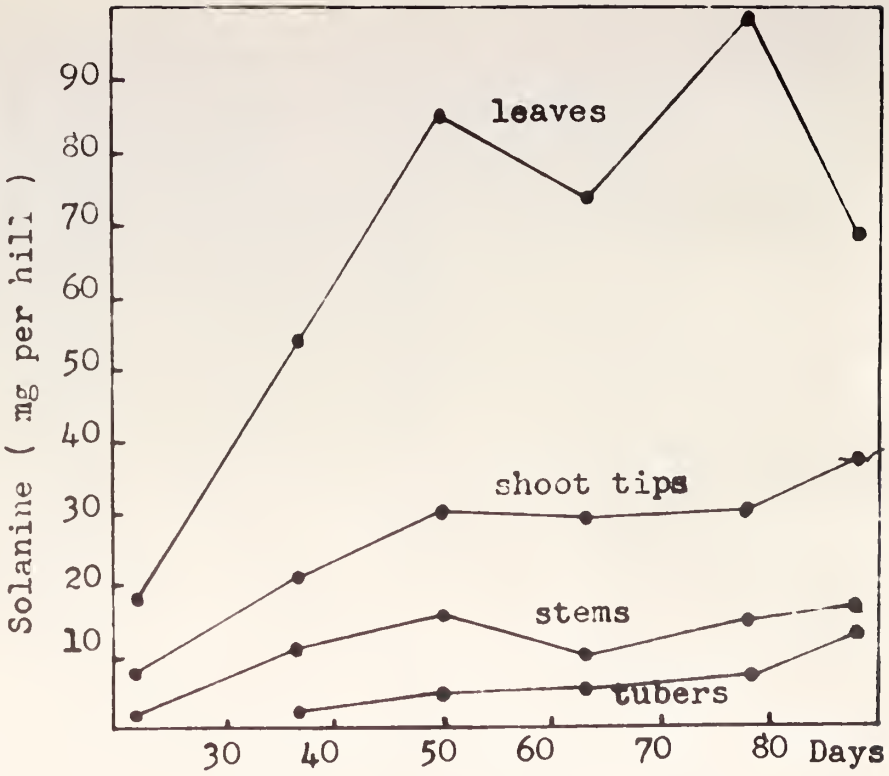


Fig. 3. Solanine content, of the developing potato plant; total per hill of various parts of Russet Burbank variety; according to Wolf and Duggar (46).

32) are believed to affect markedly the final solanine content at the time of analysis.

All these factors will now be discussed in more detail under the following three headings:

III (A)

Environment of the Growing Plant

There is little evidence of a positive nature that abnormal solanine synthesis is caused by particular climatic or soil conditions.

The general tendency of alkaloids to be destructively metabolized towards the end of the growth period (26, 46) would indicate that potato tubers at this time will display normal and minute solanine concentrations (25). The only possibility of increased content at this time may result by translocation from solanine-rich plant tops, if the natural degradation system or some natural block as suggested by Wolf and Duggar is impaired (46). This is only an assumption which needs further elucidation.

There are some indications that an upset of nutritional balance of major elements and the occurrence of hail or light summer frosts may be involved in abnormal accumulation of solanine in tubers (25).

Arutyunan reports that the incidence of high solanine content is more frequent in hot and dry climates than under temperate and humid conditions (3).

Some workers report that high nitrogen levels in the soil may result in an increase of solanine (7, 34), and also other alkaloids (13, 31). Pallman and Schindler, on the other hand, have found no apparent effect of fertilizers (N, P, K) on solanine formation (36).

The effect of cultural practices, such as dates of planting, type of hilling, size of seed and number of nodes per seed piece, was studied by Hutchinson. He could establish no correlation with the amount of solanine content (25).

The solar radiation undoubtedly must play some role in solanine synthesis, possibly in relation to photosynthesis or other metabolic process, as it has frequently been reported that sunburned potatoes are usually bitter (7, 12, 34). Such potatoes exhibit a considerable amount of greening due to chlorophyll formation, although Conner concluded that there is no definite correlation between chlorophyll and solanine formation (12).

The effect of solar radiation is seemingly associated with varying length of photoperiods. Wolf and Duggar, studying the effect of light and darkness, found that in the

presence of adequate supplies of raw material the synthesis of the glycoside is qualitatively independent of light (46). Nevertheless, they believe that light has a definite effect upon solanine distribution, as there is a tendency of light to concentrate the solanine more in the aerial position of the plant, whereas in darkness a fairly uniform distribution results.

Marked differences were found by the mentioned workers as to the distribution of the glycoside under different photoperiods. Under long-day photoperiods (18-hour exposures), about 70 percent of the solanine is concentrated in above-ground portions of the plant, while under corresponding short-day (10-hour exposures) only 58 percent of it is found in the tops. A reverse correlation was found in underground portions of the plant, and the higher amount of solanine in short-day stems and stolons may be indicative of the high rate of translocation to the tubers. Solanine content in tubers of long-day plants was found to be 9.02 mg. percent, compared to 22.70 mg. percent in tubers set under short-day photoperiods.

III (B)

Environment of the Stored Tuber

The most conspicuous increase in solanine content so far supported by experimental evidence apparently takes place during the storage life of tubers. There are implications that very little solanine is found immediately after harvest,

even though Hutchinson observed a slight increase after first autumn frosts in a relatively few cases (25).

Other evidence seems to indicate that the dormant stage of tubers may be the critical stage in which, if conditions are favorable, high solanine levels may be readily reached through induced new synthesis of the alkaloid.

Bomer and Mattis state that exposure of old potatoes to continuous light for 7 to 10 weeks did not influence the amount of solanine present to any extent, while young mature potatoes with or without the tops attached developed large amounts of the glycoside on continuous illumination (7).

On the other hand, others report that solanine content is unaffected by long storage at 4° to 5° C., though a slight rise may be noted after 6 to 7 months (46).

Against this fact we note the evidence of Naumov, who reports an increase in storage at low temperatures and at the sprouting stage, and that there is a decrease in solanine content if tubers are stored at higher temperatures (35).

This view is also supported by observations of Mayfield _et al_ which disclosed that Netted Gem potatoes stored at lower temperatures of 37° to 46° F. in a cool, damp cellar do develop a yellow-green color of tuber flesh accompanied by a very acrid flavor. This bitter flavor becomes more pro-

nounced as the storage advances (32). When tubers of the same variety are kept in a warm and dry storage cellar at 55° to 60° F. with subdued light, no indication of bitterness could be noted. Tubers of the Bliss Triumph variety did not develop a bitter flavor under either treatment. A more interesting claim by this group of workers was that solanine content for both varieties was approximately the same and after 7 months of storage reached concentrations as high as 51 to 57 mg. percent. These amounts are far above the safety limit for food purposes, and because of the unreliable gravimetric method of solanine analysis, the accuracy of these results should be seriously questioned, as mentioned earlier.

Hutchinson carried out a series of organoleptic tests of pressure-cooked Netted Gem potatoes previously stored at temperatures ranging from 0° to 20° C. (24). Some of the tubers were kept in the dark, and some exposed to subdued light. The results indicated that in potatoes kept at low temperatures the bitter flavor was maintained, or even increased, when compared to tubers kept at temperatures above 10° C.

Similar observations of Naumov (35), and Mayfield (32), as already mentioned, were partially supported by analyses for solanine present in the tubers.

Hutchinson's results reported by Hilton (22), and Mayfield's (32) observations, agree as to the peculiar

ability of Netted Gem potatoes to produce a high degree of bitterness, apparently accompanied with high solanine content.

Alexander, investigating bitter and other flavors of chips prepared from Katahdin potatoes stored at low temperatures, found that an excessive amount of reducing sugars was accumulated (1). By progressive conditioning from 1 to 90 days at 70° to 75° F., the sugar content was lowered and a sweet and burned flavor observed earlier had disappeared while the bitter flavor persisted in many lots.

The fact that low storage temperatures bring about an increase in solanine content points toward some unidentified internal factor or factors involved in complex metabolic changes such as enzymatic hydrolysis of starch to sugars under these conditions.

Bonner and Arreguin studied extensively such internal changes in potatoes with regard to starch synthesis (8). Results from their work which are pertinent for reproduction are shown in Table 1. Potato tubers at temperatures above 10° C. retain their starch, while at lower temperatures starch is rapidly lost and sugars accumulate, particularly sucrose. Changes in the phosphorylated compounds as illustrated in this table indicate that starch degradation at low temperatures involves complex transformations of phosphorylated sugars. It was also found that at high temperatures an in-

hibitor of phosphorylase, the starch synthesizing enzyme, is accumulated, and that this inhibitor disappears at low temperatures to enable increased activity of systems capable of withdrawing glucose-1-phosphate for other syntheses (8).

Table 1. Changes in sugars and sugar derivatives in potatoes stored at various temperatures (according to Bonner and Arreguin (8).

Substance	Initial content	Amount in % dry wt. of tissues after 2 weeks at temperature indicated			
		0°	9°	16°	25°
Starch	67.0	61.0	65.0	63.0	64.0
Glucose	0.62	0.79	0.73	0.49	0.56
Fructose	0.17	1.50	0.34	0.22	0.15
Sucrose	1.07	6.65	1.25	0.75	0.84
Glucose-1-phosphate	0.00	0.17	0.04	0.00	0.00
Glucose-6-phosphate	3.50	0.70	0.66	4.20	4.50
Fructose-6-phosphate	0.17	2.50	1.05	0.25	0.35
Triose phosphate	0.37	0.94	1.07	0.57	0.26

Besides the conversion of starch to sugar and *vice versa*, there are other metabolic processes taking place, the most important being respiration and transpiration. With regard to the former, potatoes exhibit a very peculiar behaviour. Lowering the temperature from a few degrees above

0° C. to about 0° C. results in an increase of respiration, which is due to the shift of starch/sugar ratio towards sugar formation and thus towards an increase in respiratory substrate. Also light (which is found to have a definite effect at these temperatures upon solanine formation) may have an indirect effect upon respiration as a carrier of radiant energy (33).

Thus, it is not excluded that the transformations of phosphorylated intermediates and the increased amount of respiratory energy, in potatoes at low temperatures, may be utilized in solanine synthesis. The findings of Naumov (35), Hilton (22) and Mayfield (32) appear to substantiate this postulation.

III (C)

The Effect of Light Quality and Intensity upon Solanine Synthesis

In earlier discussion, light has been considered to play an important role in solanine synthesis. Thus, greening of tubers caused by exposure of unprotected tubers at soil surface to sunlight, and to artificial illumination in subsequent storage, has been found to be correlated with high solanine content and the resulting bitterness. Von Morgenstern was first to note that tubers which had turned green on exposure to sunlight contained higher-than-normal concentrations

of solanine (34). Bomer's and Mattis' experiment with exposure of young and old mature tubers to continuous illumination has also been mentioned (7).

Arutyunan reports an increase of the alkaloid content under exposure to sunlight and ultra-violet light (3). Lepper made similar conclusions, reporting a slight increase of solanine when tubers are exposed to sunlight; however, following a period of storage solanine content is again decreased (30). In the field, "sunburned" tubers are reported to have solanine content as high as 58.8 mg. percent (17).

The effect of individual components of the visible and ultra-violet spectrum of radiant energy was extensively investigated by Conner (12). He attempted, using potatoes of Bliss Triumph variety, to determine whether equivalent light intensities under the 6900 Ångstrom limit, effective in glucose and chlorophyll synthesis, are also efficient in solanine formation. As similar investigations are to be reported here, Conner's work will be given detailed consideration, and his conclusions are summarized as follows:

(1) Wave-lengths which are efficient for glucose synthesis did not induce significant increase in solanine formation, but did result in chlorophyll elaboration.

(2) Upon irradiation by bare mercury arc, potato tubers considerably increased in solanine content, which was accompanied by anthocyanin

formation in the sprouts.

(3) Ultra-violet rays of the 3000 Ångstrom length are effective in solanine formation, but not for chlorophyll synthesis.

In sunburned potatoes, after four months of storage, Conner found surprisingly low solanine concentration of 3.1 mg. percent only, and on exposure to bare arc 10.8 mg. percent. With a relatively intense ultra-violet light source, solanine content was only 4.7 mg. percent at most. These low results, as shown by Rooke et al (39) are due to the fact that Conner's hydrolytic method determines solanidine only, and not the "total" solanine content.

An important factor which was more likely to affect solanine than chlorophyll content, and which Conner did not take into consideration, was the radiant energy beyond his 6900 Ångstrom limit. From his graphically presented spectral energy distribution of each light source and the Corning glass filters used, it appears that relatively large amounts of energy were supplied from the zone of the infra-red spectrum. This was the zone which Conner erroneously assumed to have little effect upon solanine synthesis. As will be shown later in this report, the radiant energy beyond the visible spectrum had a significant influence.

Thus, it may well be that light energy, which is of vital importance in photosynthesis, uptake of nutrients

(in water plants), chlorophyll formation and other photochemical processes (8, 33), is involved in, and possibly essential for, such a complex intermediary metabolic process as solanine synthesis.

In the foregoing discussion of certain published reports pertaining to the solanine problem, the important internal and external factors that might play a role in excessive solanine accumulation in potato tubers have been delineated.

Taking into consideration recent biochemical achievements relating to genetic and physiological aspects of biosynthesis in living plants, and views as to other alkaloidal syntheses, the following review is presented.

IV

General Aspects of Biosyntheses in Plant Tissues and Biogenesis of Tropane Alkaloids

Beadle reviewed and summarized in his reports the latest investigations of genic interactions and biosyntheses of complex organic substances produced by a variety of living plants (4, 5, 6). Evidence was compiled that genes direct almost every vital metabolic process, not only in animal, but also in plant tissues (5). Although Beadle's review is not directly concerned with solanine or alkaloidal synthesis, useful information is given as to the interrelation of factors

or processes which may be involved; and, furthermore, it demonstrates the complexity of the problem to be dealt with.

Glucoside formation in anthocyanin pigments is known to be genetically controlled in at least two cases. In species *Verbena* and *Streptocarpus*, the formation of 3,5-dimonoside is *genetically* different from a 3-monoside type of anthocyanin (4).

The quantitative aspect of tobacco alkaloid inheritance, which has received much attention lately, has been reviewed by Dawson (13). It is now well-known that strains of Burley tobacco were bred that contain exceedingly low nicotine and total alkaloid content, while *Nicotiana rustica* strains were selected in which exceptionally high yields of nicotine are produced. In view of the assumption that genes, directly or indirectly, through enzymes, govern biosynthesis in living tissues (5), there is little doubt that genic dissimilarity accounts for high and low alkaloid yielding strains of tobacco (13).

Studies of inherited ability of mutant *Neurospora* (breadmold) strains to synthesize specific vitamins of the B group have been reported (6). Horowitz, investigating four radiation-induced gene mutations that differed only in a single gene, found that such mutants are unable to synthesize the essential amino-acid methionine (23). In each of these, the methionine synthesis is blocked in a single step which is thus

under genic control, and as a result the metabolite produced in the preceding step is accumulated. This was illustrated by Horowitz as follows:

$$\xrightarrow{\text{gene me-4}} \text{cysteine} \xrightarrow{\text{gene me-3}} \text{cystathione} \xrightarrow{\text{gene me-2}}$$

$$\longrightarrow \text{homocysteine} \xrightarrow{\text{gene me-1}} \text{methionine}$$

Also in Saccharomyces strains, it was found that the ability to synthesize panthotenic acid is differentiated by a single gene (6).

Numerous metabolic processes in animal systems have been known for a long time to be directed by genes, and only recently a similar view was substantiated in the plant world in such instances as just mentioned. The older view that genes act by controlling enzyme specificities was supported impressively in recent years (5).

By virtue of their auto and hetero-catalytic character, genes are directing specific configurations of protein molecules, and thus directly determine enzyme specifities. Thus a primary control of enzymic syntheses and biogenesis of organic compounds in living tissues is attributed to genes (4).

Green states that intermediary metabolism of living tissues is carried out by virtue of myriads of chemical reactions, which are not spontaneous (18). Highly specialized protein catalysts known as enzymes are indispensable for a highly organized living system, as practically every reaction is under the control of a specific enzyme. The essentiality of nutritional elements is also definitely linked with the enzyme system, and thus indirectly with intermediary metabolic processes, as most of such enzymes are closely correlated with micro-elements such as copper, zinc and others. Green also advances the enzyme-trace theory which suggests that any substance, regardless of origin, that produces biological effects in trace amounts does so either by participating in, or by affecting, some specific enzymic system. Vindication of this theory was achieved in at least fourteen instances. It has been found that some vitamins are actually functioning as prosthetic groups of enzymes, and four authentic identifications of vitamins with prosthetic groups were accomplished (B_1-thiamin, B_6-pyridoxal, B_2-riboflavin and nicotinamide).

Evidence compiled and presented by Beadle and Green is undoubtedly in favor of gene or enzyme-controlled biosyntheses of intermediary metabolism from which, at least in certain plant species, such compounds as alkaloids may arise. The mechanism involved as yet is not understood.

The biosynthesis of Belladonna alkaloids in three solanaceous species - Datura, Atropa and Hyoscyamus - was extensively studied by James (26, 27). These alkaloids appear at an early stage of germination, although the seed and embryo are devoid of them. By cytochemical investigations, it was shown that roots of germinating seedlings up to 3 millimeter length are completely alkaloid-free, and on further elongation copious amounts of tropane alkaloids appear, the place of their synthesis being actively growing tissue only, i.e. meristem proper (27). Towards the end of the vegetative growth period, a reverse process apparently takes place as degradation of alkaloids is noted and they disappear from the tissues (26).

This view seems to be supported by Wolf's and Duggar's investigations of seasonal changes in solanine content of various parts of the developing potato plant as reproduced in Figure 2 (46). The total solanine content is at its peak early in the season and gradually decreases towards the end of it. It was found that a total of 284 mg. of solanine disappeared from the aerial portion of the White Rural variety. The theory that this disappearance is due to metabolic destruction is supported by the finding that only 13 mg. percent of solanine was present in the tubers, whereas any translocation from the tops would result in at least 71 mg. percent. The view of the investigators that some natural block prevents

any possible translocation is substantiated by Morgenstern's observation that brown wilted potato leaves at the end of the season contain free solanidine (34).

It is also interesting to note that alkaloid synthesis is closely related to protein synthesis. In view of the well-known effect of potassium upon protein synthesis, the apparent retardation of alkaloidal synthesis, as discussed by James, affords an interesting suggestion of competition between the two processes for available nitrogen (26).

With respect to solanine itself, high levels of nitrogen are believed to increase its amount in potato plants, while potassium has no apparent effect (34). Nitrogen fertilization was found to promote to some extent nicotine synthesis (13) and formation of Cinchona (31) and Belladonna alkaloids (26). Detached leaves of Belladonna spp. fed with ammonium sulphate and sucrose in the dark have been shown to increase their total alkaloid content per leaf more vigorously than leaves fed on sucrose only (26).

A close consideration should be given also to a study of the nature and distribution of various forms of nitrogen in the potato plant as reported by Street et al (41). Solanine is considered as a quantitatively unimportant part of the "mobile" nitrogen. The mobile forms include purine, adenine and guanine nitrogen, polypeptide and peptide nitrogen, simple natural bases such as choline and trigonelline, and

non-amino nitrogen of arginine, proline and tryptophane. Simple natural bases are of special interest in view of their universal occurrence and possible significance in alkaloidal synthesis and biological methylation.

With regard to solanine concentration, the results of these workers agree with other reports (28, 46). A high rate of solanine synthesis at the beginning of the season was noted especially in leaves and roots, while the gradual decrease towards the end of the season was clearly established. Flowers had solanine content as high as 618 mg. percent of fresh weight.

The evidence presented in the foregoing review disclose the complexity of the potato bitterness problem as caused by abnormally high solanine synthesis and its translocation and accumulation in the tubers.

The internal factor or factors which are seemingly maintaining the alkaloid level within limits of varietal differences and seasonal variations appear to be considerably affected by external environment.

There is no evidence as to whether abnormally high concentration of solanine in the tuber is a result of a synthesis in situ, or whether it is due to translocation from solanine-rich aerial portions of the plant.

EXPERIMENTAL DESIGN

General Layout of Experiments

Due to the necessity for a large number of repetitive analyses for solanine when statistically laid-out trials are used to test variables, such a procedure limits seriously the number of factors that can be investigated. For this reason, and because of the fact that solanine analysis is a time-consuming, arduous procedure, the experiments were designed to explore a large number of possible causal factors and conditions instead of being carried out on a statistical basis.

The complexity of solanine synthesis, as reviewed earlier, necessitates a rather fundamental approach to search the factors or conditions responsible for, or affecting the amount of, alkaloid developed in plants and tubers. There is very little of positive nature known about these conditions, and more experimental data and evidence are needed to support any general conclusions.

The investigation was carried out along four rather independent lines. Under field, greenhouse and storage conditions, various treatments were applied to plants and tubers so as to note their effect upon solanine occurrence. In addition, the ultra-violet and infra-red components of the radiant energy spectrum were explored as to their effective-

ness in solanine synthesis of potato tubers during and after irradiation.

Two potato varieties were used in the investigation; one, Netted Gem, is very often reported to develop a high degree of bitterness; and the other, Katahdin, is usually less frequently found to be bitter.

Occasionally the solanine content of a third variety, Carter's Early Favorite, was investigated because high solanine content was found in a sample sent from Vermilion district.

Greenhouse experiments and irradiations were carried out with the Netted Gem variety only. Also, for observation, potato seed with unusually high solanine content (47.2 mg. percent), secured from a St. Albert producer, was planted on the field. Otherwise, the planting material was provided from regular University of Alberta potato seed stock.

The experiments were designed as follows:

1. Field Experiments.

Approximately a total of 1,500 potato plants of earlier mentioned varieties were grown in the 1952 season in a field plot in rows of east-west direction, 3 feet apart, the spacing of plants in a row being 1 ft. The plants made good growth until near the end of the season, when 6 differing treatments were applied, in attempts to study the effect of

these upon the behaviour of alkaloidal content in harvested tubers.

The experimental treatments, as noted in detail later, were applied at random to parts of rows to provide a sufficient number of samples for chemical analysis. At a certain time interval after the treatment, single treated hills were dug and prepared for solanine analysis, as described later.

2. Greenhouse Experiments

A few potted Netted Gem potato plants were grown under greenhouse conditions at the same time as plants in the field plot. Only 3 to 4 plants were used for each treatment, some of which were similar to those in the field whereas others included exposure to infra-red and ultra-violet light.

3. Storage Conditions

In attempt to study the effect of various storage conditions, tubers of Netted Gem and Katahdin varieties were exposed to four different treatments, the influence of which was to be correlated with solanine content as determined by analyses at weekly intervals. In order to compensate for loss of tuber weight due to transpiration and respiration, the tuber weight for each sample was recorded at the beginning and end of treatments, and the solanine content was eventually recalculated to mg. percent of untreated tuber tissues.

Tuber samples were exposed to a weak Mazda light, or kept in complete darkness under two conditions as follows:

(a) Normal cold and relatively humid storage at temperatures from 4° to 8° C.

(b) Warm and dry storage with temperatures from 12° to 15° C.

4. Irradiation of Tubers Using Ultra-Violet and Infra-red Light

Numerous reports indicate that ultra-violet light is effective in solanine synthesis, and no work has taken into consideration the infra-red zone of radiant energy spectrum that is beyond the visible light spectrum. In order to compare the effectiveness of both sources of energy, active and dormant tubers of Netted Gem variety were irradiated for various time intervals.

As infra-red light emanates considerable heat, a special cooling device with a continuous flow of tap water, with occasionally added ice cubes, was assembled in order to lower the temperature at the irradiated face of tubers.

Because of considerable loss of tuber weight on exposure to infra-red light, the loss of weight has been determined in order to enable recalculation of solanine content to original weight prior to the treatments.

The physiologically active material was secured from the previous season's crop, unfortunately after 8 to 9 months of storage, and consequently with considerable amount of sprouts formed. The "dormant" material was secured from the new crop harvested from plants grown in the field plot.

Methods of Sample Preparation for Solanine Analysis

Analytical samples of the field and greenhouse experiment were represented by tubers of a single hill or a pot in which plants were grown. It was assumed that variations in solanine content of such samples would be negligible. All tubers protruding from the soil and thus exposed to light, usually showing traces of chlorophyll, were discarded.

From each of these samples, 6 to 8 normal and uniform tubers were selected for analysis, very small or damaged tubers being excluded and discarded. Only one-half of each of the selected tubers, cut through the longitudinal axis, was finely ground and minced in a Waring blendor for exactly 5 min. after the pulp began to circulate. The other tuber half was discarded. Thus, the resulting pulp was believed to represent an accurate blend or mean of the solanine content per single hill or plant.

In order to prevent undesirable enzyme action, the pulverized tuber tissue was weighed in duplicate 25-gram samples in small tin cans, immediately quick-frozen and stored

in a deep-freeze storage at -22° C.

To provide replacement material for occasional cases when analytical procedure would yield inconclusive and variable results, a part of the ground tissue was quick-frozen and stored in 1/4 pt. waxed paper containers.

At a convenient time, when analysis was to be carried out, samples were quickly defrosted and prepared for extraction.

In storage and irradiation experiments, three or four uniform large tubers were exposed to single treatments, at the end of which period they were likewise prepared for analysis.

Analytical Procedure for Solanine Determination

A critical review of analytical methods for total solanine content was presented by Dabbs (14). His most valuable contribution was the improvement of earlier cumbersome and time-consuming extraction methods. This method, which was used in the present investigation, is carried out in two steps, as follows:

1. Extraction of solanine with acidified ethyl-alcohol using the Soxhlet extraction apparatus; and

2. colorimetric determination of extracted solanine in weak acid solution, a modified form of that described by Pfankuch.

1. Extraction Technique

The defrosted 25 gm. samples of potato tissue are quantitatively transferred and washed (using 95% ethylalcohol) into a Soxhlet thimble (80x33 mm.), which is carefully placed in the apparatus. A further 100 ml. of 95% ethylalcohol are added to the distilling flask and acidified with 0.1 ml. of glacial acetic acid.

The extraction apparatus is then assembled on a series of hot plates adjusted to a temperature that would just permit the alcohol boiling (about 80° C.). The extraction is continued for a minimum of 20 hours.

The final extract in the distilling flask is then reduced in vacuo over a water-bath to approx. 5 to 10 ml. volume. A few ml. of 0.5 percent acetic acid are used to wash the rubber stopper with a fitted capillary tube, and the distilling flask is then vigourously shaken in order to dissolve or remove any brown precipitate adhering to the walls.

One gram of anhydrous sodium sulphate is added and the extract is next digested on a water-bath at 70°- 75° C. for 15 to 20 min., until a flocculent precipitate, most of protein material, is formed.

The extract is cooled to room temperature, 0.5 ml. of 20% sulphuric is added, and after thorough shaking it is filtered through a Whatman's #4 filter paper; the protein precipitate is then thoroughly washed with 0.5% acetic acid, keeping the volume of filtrate as low as possible in order not to exceed 2/3 of a 50 ml. beaker volume. To the protein-free extract, 5 ml. of conc. ammonia is added immediately to prevent any undesirable reactions which may take place at low pH.

With the aid of a Beckman pH-meter, the extract is then adjusted to a pH of 10.0 to 10.4 (with concentrated ammonia), and digested again on a water-bath at 70° C for 25 to 30 min. A flocculent precipitate of solanine and, if present, of solanidine is obtained. The extract with the precipitate is then cooled at least for some hours in a refrigerator, or preferably left there just overnight.

The solanine is then filtered off through a #42 Whatman's filter paper and washed thoroughly with 2% ammonia water long enough to remove completely the brownish color which results, possibly, from intermediates of melanin formation.

The filter paper with the precipitate is then left standing until completely dry, with no smell of ammonia remaining, and placed back into the same beaker in which earlier solanine has been precipitated.

The beaker, which has some crystalline solanine left on the sidewalls, and the filter paper can then be stored

until colorimetric procedure may conveniently be carried out.

2. Colorimetric Determination of Total Solanine

The solanine precipitate on the filter paper is placed first on a clean funnel, dissolved and washed with small portions of 0.5% acetic acid into a 50 ml. volumetric flask. The crystals in the original beaker are likewise dissolved in small amounts of dilute acid and filtered through the same filter paper, which is washed with successive increments of the acid. The volume is finally made up exactly to 50 ml.

From each representative sample solution, 3 ml. aliquots are measured with a precision pipette into a clean 50 ml. Erlenmeyer flask. From a suitable microburette, 6 ml. of C.P. sulphuric acid reagent is added drop-wise to the flask, which is being whirled and cooled in an ice bath. After the addition of the last drop of sulphuric acid, the solution is left standing for 1 minute and 3 ml. of freshly prepared 1% formaldehyde is likewise added drop by drop.

Presence of solanine in the mixture with sulphuric acid is indicated by a greenish-yellow color which, on addition of formaldehyde, changes to light orange and finally a definite cherry-red color, the maximum intensity of which is reached after 45 to 50 min.

Colorimetric determinations of color intensity are then made using 3 ml. micro-tubes on an AC-model Fisher

electrophotometer. A green color filter B-525 is used.

The analytic method is described in detail because of some slight modifications which were found convenient. The addition of sulphuric acid was found convenient to be carried out by means of a Koch-microburette, commonly used in blood analysis. This burette has a ground-glass stoppered reservoir attached, which prevents any dilution of the very hygroscopic acid, and has a special exchangeable tip which allows a very steady rate of drops.

The calibration factor for the particular electrophotometer used was determined by the use of two standard solutions of 25 mg. C.P. solanine per 250 ml. of 0.5 percent acetic acid. From these standards a series of dilutions was prepared to obtain 2 series of 8 solutions, containing 1 to 8 mg. of solanine per 50 ml., each dilution differing from the next by 1 mg. solanine present.

The solanine prepared and purified by Dabbs (14) was used. The factor of conversion for the electrophotometer was calculated as follows:

$$F = \frac{\text{mg. solanine in standard solution}}{\text{"A" reading of photometer scale}}$$

The sum of factors for each standard dilution divided by the number of dilutions yielded the average value of F = 0.228.

Each reading, then, which is given by an unknown 50 ml. solanine solution obtained from a 25 gm. potato tuber sample, multiplied by fourfold value of F (0.912) represents the amount of milligrams of solanine per 100 gm. of tuber tissue.

This value includes solanine and, if present, solanidine, generally designated as "total solanine." All presented data of this investigation refer to "total solanine" expressed in milligrams per 100 gm. of fresh weight of tuber tissue, or, as sometimes expressed, "mg. percent."

EXPERIMENTAL RESULTS

I. Field Experiments

For reasons described earlier, the experiments were designed to explore the largest number of possible factors, and therefore any statistical layout was omitted. A series of treatments was applied to growing plants at the end of the growing season, tuber samples being taken at one or two-week intervals after the treatments. For convenience, as there was little correlation between various treatments, each of these will be presented and discussed independently.

In order to provide sufficient material for the tests, a large field plot of potatoes was grown, and for each treatment a part of a row of uniform plants was selected at random. Sixteen rows, 90 ft. long, were seeded with three varieties, the rows running in east-west direction, as follows:

Carter's Early Favorite	-	3 rows	with	approx.	270	plants
Netted Gem II	-	1 row	"	"	80	"
Katahdin	-	5 rows	"	"	450	"
Netted Gem I	-	7 rows	"	"	630	"

All these varieties will be described in subsequent tables pertaining to each single experiment as CAR, NGII, KAT and NGI.

Netted Gem I was from the University of Alberta variety stocks, and the seed material had a normal solanine content, while Netted Gem II was secured from a St. Albert producer's stock potatoes with abnormally high solanine content

of 47.2 mg. percent.

Multiple-eye 1½ to 2 oz. seed pieces were planted on May 26, 1952, into rich Edmonton black clay-loam, in which a good crop of beans was grown the previous season.

No cultivation, except occasional tilling to keep the plot free of weeds, was carried out, the plants growing vigorously and freely until early fall when first treatments were applied. Roguing for seriously diseased and virus-infected plants was carried out frequently. The first treatment was applied on August 25, after 92 days of growing period. The area occupied by the entire plot had been manured in 1950 and appeared to be uniform in physical respects.

Experimental treatments were carried out as listed:

1. Chemical vine-killing with aerocyanate.
2. Mechanical removal of the vines.
3. Mechanical damaging of vines.
4. Harvesting at 3-day intervals after first autumn frosts.
5. Exposure of hills to direct sunlight by bending the tops and harvesting tubers from various depths.
6. Exposure of tubers to solar radiation at harvest.

Each of these treatments is described and discussed independently immediately below.

1. Chemical Vine-killing with Aerocyanate

There are many recently published reports pertaining to chemical vine-killing, which is an important cultural practice of recent development. Papers deal particularly with the possible effects on tuber quality, in case some toxic chemicals may be translocated to the tubers.

In order to obtain information as to whether there is any effect upon increased solanine translocation to the tubers or increased synthesis of this alkaloid after vine-killing, field treatments were applied to fully grown plants near the end of the vegetative season. The killing was carried out at two dates with a spray of 1 pound of calcium aerocyanate per gallon of water at a rate of 1 pint per 6 to 8 uniformly developed plants, to provide sufficient material for 4 to 5 replicate analyses. Plants were selected at random from a row of each variety grown. The first spray was applied on August 25 and the second spray 8 days later, the single hill samples (controls) being taken at the time of treatment and at 8 and 16 days following the spray.

The treatments were at first to be applied two weeks apart; however, because of the very cool weather conditions at the end of August, and threatening frost which might have invalidated the second treatment, the second spray was carried

out one week after the first.

Average solanine contents for each treatment are given in Table 2.

The total weight of tubers for each sample-hill was recorded, but as no significant correlation could be established between the yields and solanine contents, the presentation of cumbersome yield data will be omitted.

All estimates of solanine content are given in milligrams per 100 grams of fresh tuber tissue weight.

The results of chemical analysis show all samples with an unusually low solanine content, regardless of treatment effects, and it is obvious that the treatment had no apparent effect upon solanine content. The solanine concentrations of both Katahdin and Netted Gem potatoes are much lower than those given as normal under Wisconsin conditions (5.5 and 4.7 mg. %, respectively) (46). Only in one Katahdin and two Carter's samples did the concentration exceed 3 mg. %. This fact might be due to two factors, one being that samples for analysis were prepared and quick-frozen not later than 1 and 2 weeks after harvest and therefore no interfering effect of subsequent storage could have influenced the solanine content. The second factor is that solanine content at the end of the growing season often is very low, and this finds support from Hutchinson's work (25), where samples of the 1951 season, from

Table 2. Average solanine (mg. % of fresh weight) in tubers of three potato varieties as affected by the vine-killing treatment.

Variety	NG I		KAT		CAR		NG II	
Days after treatment	8	16	8	16	8	16	8	16
(a) First spray on August 25, 1952								
Control	2.05	2.51	2.14	1.87	3.06	1.78	1.96	2.37
Treated	1.72 (5)*	1.55 (2)	2.61 (5)		2.75 (4)	3.78 (2)		
(b) Second spray on September 2, 1952								
Control	2.51	2.01	1.87	1.49	1.78	3.29	2.37	
Treated	1.75 (5)	1.09 (2)	1.55 (3)	1.59 (2)	2.85 (3)		2.60 (2)	

* Figures in brackets refer to numbers of samples analyzed. Each control represents an average of two samples.

the same field conditions and from vigorous healthy plants on September 3, showed only traces of solanine or none at all.

In the above connection, Wolf's and Duggar's observation is of interest; i.e., that solanine in the vines is destructively metabolized, and little translocation to the tubers will take place (46). Another fact which favors this idea is that negligible amounts of solanine in the plants originated from seed with abnormally high solanine of 47.2 mg. %; and in the plants at the end of the season, the solanine concentration exceeded 3 mg. % in one case only.

Thus it would appear that solanine occurrence is more influenced by subsequent storage conditions than by translocation from the tops, as might be assumed to occur, and it is also doubtful that possible translocation was prevented by the treatment.

More information as to this fact might be obtained by treatment at later dates, and by allowing the tubers to be stored for longer intervals after treatment under conditions which do not favor solanine synthesis.

2. Mechanical Removal of Vines

The study of solanine distribution reveals that most of the glycoside is concentrated in the aerial portion of the plant, mainly in the leaves, flowers and fruits. Also,

it is frequently suggested that under conditions which would upset metabolic processes solanine may be translocated to cause higher-than-normal solanine concentration in the tubers.

To investigate the effect upon solanine level of vine removal from the plants at the end of the season, the plant tops were cut with a sickle on two dates, August 25 and September 2, tuber samples being dug at the time of treatment and one week later in each case.

The solanine contents as found in analyzed tuber samples are presented in Table 3, and are expressed in milligrams percent of fresh tuber weight.

Table 3. Average solanine content in potato tubers (mg. % of fresh weight) as affected by the mechanical removal of vines.

Variety	NG I	KAT	CAR	NG II
	(a) First treatment on August 25			
Control	2.05	2.14	3.06	1.96
Treated	1.79 (5)*	1.09 (4)	2.22 (4)	3.22 (3)
	(b) Second treatment on September 2			
Control	2.51	1.87	1.78	2.37
Treated	2.00 (4)	1.86 (4)	3.01 (4)	

* Figures in brackets refer to numbers of samples analyzed.

Each control represents an average of two samples.

The results of this treatment are similar to those of vine-killing, generally very low and exceeding a concentration of 3 mg. % only in two Carter's Early Favorite samples and one of the Netted Gem II samples.

The same conclusion has to be made that the treatment at this time of the season had no apparent or immediate effect upon solanine content, and that due to the fact that no long storage was allowed very little of the glycoside was actually found in the tubers. Two weeks of storage, at the most, was allowed prior to preparation of samples, and more information would be at hand if the effect of this treatment could be followed through subsequent storage.

3. Mechanical Damaging of Vines.

Hail and frost damage have been reported in Alberta as coincident with the occurrence of bitterness in potatoes (25). It was assumed that this type of damage causes an upset of physiological processes in the plants, and that it might facilitate release and translocation from the solanine-rich tops to the underground portions of plants.

In an attempt to test this possibility, a few plants from each variety were treated by simply crushing and beating the vines with a stick in such a way that damaged vines still remained attached to the roots and were not completely killed.

The treatment was carried out on the same two dates as previous experiments, and potatoes from each hill were dug separately at the time of treatment (controls), and 8 and 16 days after treatment application.

Experimental results are shown in Table 4.

Table 4. Average solanine (as mg. % of fresh weight) in tubers of three potato varieties following mechanical damage of tops.

Variety	NG I		KAT		CAR	
Days after Treatment	8	16	8	16	8	16
(a) First treatment applied on August 25						
Control	2.05	2.51	2.14	1.87	3.06	1.78
Treated	2.78	1.35	1.87	-	2.21	1.82*
(b) Second treatment applied on September 2						
Control	2.51	2.01	1.87	1.49	1.78	3.29
Treated	2.13	1.92*	-	1.37	1.78	2.47

* All figures represent the average of two samples except those two marked with an asterisk, in which case only one sample was analyzed.

The results of this experiment again indicate no apparent effect of the treatments upon solanine content of the tubers. Although there was a tendency in Netted Gem variety to exhibit a higher content shortly after the first treatment,

the samples taken after the second treatment one week later showed a lower content compared to control samples. In Carter's Early Favorite tubers, there is a slight decrease of solanine 8 days after the first, and 16 days after the second, treatment. Such a decrease is, however, of very little significance, and is probably entirely due to variability between single plants.

It is thought that the damage to the plants was rather serious, and the interval between treatment and taking of samples too short (1 week only). A better estimate of effect might be achieved by less serious damage to the plants earlier in the season, thus allowing a better "incubation" period until the samples are dug for analysis in late fall. If the supposition is true that frost and hail damage may result in increased transportation and accumulation of the glycoside in tubers, it is possible that slight damage and sudden drop of temperature just above the soil surface may be the responsible factors if early frosts or hail occur. This might be tested by utilizing cracked ice along with very slight damage to vines.

4. Harvesting of Tubers at Three-day Intervals After First Autumn Frosts.

Hutchinson noted in previous season a slight increase in solanine content (in 1951), following severe frost, while no solanine was found in tubers dug prior to the frost

(25). Similar observations were made by Street et al (41), and Wolf and Duggar (46), as they found a decrease in solanine content of plant tissue and of the tubers towards the end of the season.

Therefore, when the first fall frost occurred on October 3, tubers were dug for control, and subsequently at intervals of a few days samples were taken in order to determine the effect of frost upon the occurrence of the alkaloid. Analytical results are presented in Table 5.

Table 5. Solanine content in tubers of three varieties after first autumn frost

Days after frost	NG I	KAT	CAR
1	1.64*	1.14	1.69
3	2.74	1.46	2.65
6	1.69	1.46	--
9	1.64	1.78	1.36
13	1.87	1.92	2.14
16	2.03	2.33	--
20	1.87	1.39	--

* Average of three duplicate sample analyses; the remaining figures are averages of two tuber samples.

The glycoside concentration was repeatedly found to be exceedingly low, and in only two samples the total amount exceeded 3 mg. %. In the Katahdin variety, only 3 samples out of 14 showed a solanine content exceeding 2 mg. %, and in the Netted Gem variety the picture was much the same. Additional determinations showed that there was no difference between plants which had dry mature, dead vines prior to the frost, and those plants which were actually killed.

Thus, the earlier observation of Hutchinson (25) does not seem to be confirmed, as he reported solanine contents as high as 8.28 and 6.65 mg. %, respectively, shortly after the frost-killing in the 1951 season occurred.

A possible explanation for the exceedingly low concentrations of the alkaloid may be that the frost-killing was too sudden and severe. The majority of those plants which were green and active were instantly killed, and any translocation of the glycoside from the tops was presumably prevented. Had a lighter frost been experienced, possibly Hutchinson's observation may have been borne out.

5. The Effect on Solanine Build-up of Exposing Hills to Direct Sunlight

As greening of tubers, and exposure of tubers to daylight or artificial illumination, was often reported to be associated with increased production of solanine, an attempt

was made to determine the influence of such solar radiation as might penetrate the soil to various depths under field conditions.

In this experiment, the green potato vines at the end of the season were bent to one side and weighted there so as to expose one side of the hills to direct sunlight, and thus facilitate penetration of light rays, if any exist. An attempt also was made to measure the amount of light penetration. Following the treatment, tuber samples were taken from various depths ranging from 1 to 4 inches, and the solanine content of tubers at each depth was determined.

The bending of tops was carried out on August 25 on 25 hills of a Netted Gem row, a similar part of a next row being kept as control, with no treatment.

Because of unfavorable weather conditions and little sunshine during the following week, the experiment was extended to 23, instead of a proposed eight, days; i.e. until September 18.

After this time, the vines were carefully removed and the soil covering the tubers was gradually pushed aside so as to collect potatoes at 1, 2, 3 and 4 in. depth, separately from each depth.

It was also attempted to assess quantitatively the amount of light penetrating into Edmonton clay-loam to the

depths from which tubers were collected. Because of the lack of suitable photocells and other electrical measuring equipment, it was decided to use Kodak spectroscopic photoplates, buried at given depths. In order to protect the emulsion of plates against undesirable moisture effect, these were wrapped in cellophane and assembled in a small wooden box to the depths at which measurements were desired. The assembling of plates had to be carried out very carefully in a dark-room, and the plates were then covered with soil taken from the experimental field plot. The prepared box was then placed at night between a row of field plants at soil surface level and left unprotected during the following week. Examination of the plates after one week of exposure showed that, in spite of protecting cellophane, there was some swelling of the photo-emulsion, caused by some diffusion of the soil moisture. After the failure of another such experiment, this procedure was dismissed, since due to the inadequacy of the equipment no practical conclusions were possible.

Although light is believed by many workers to play an important role in solanine synthesis, it is doubted that under field conditions the amount of light which possibly penetrates does have any appreciable effect.

The analytical results of a total of eight control samples from unexposed hills, and eight treatment samples from exposed hills, are presented in Table 6.

Table 6. Solanine content of Netted Gem tubers taken from different soil depths after bending plant tops to expose the soil to direct sunlight.

	Depth from which tubers were collected in inches			
	1	2	3	4
Unexposed	1.58	1.72	1.47	1.19
Exposed	2.74	2.19	1.55	1.71

The solanine concentrations were very low, as were those in all previous treatments. Here, however, there is a clear tendency towards higher concentrations in tubers closer to the surface, if the soil is unprotected and exposed to sunlight. At the three-inch depth, there is an insignificant difference between tubers from unexposed and exposed hills, but at the other three depths a fairly substantial increase is noted where the hills were exposed. No statistical analysis could be attempted on this sparse data.

It would be desirable to extend the exposure of hills for a longer time and follow the behaviour of solanine also in subsequent storage, in order to determine whether tubers with slightly higher solanine content at the end of the season are able to synthesize the glycoside faster than those with a lower content.

6. Exposure of Tubers to Solar Radiation at Harvest Time

It is a common practice, when potatoes are being harvested from wet soil, to spread them out on the soil surface to dry by exposure to sunshine, prior to transfer to normal storage. It seemed desirable to obtain information as to what extent the solanine content may be altered by such a treatment.

The main harvest of potatoes from the untreated field plants was made on October 10, 1952. From these, a total of seven samples of each of the Katahdin and Netted Gem varieties were collected, two of which were immediately quick-frozen for analysis as controls. From the remaining samples, two of each variety were exposed to solar radiation for 6 and 12 hours, and one sample for a total of 36 hours to solar radiation when weather conditions were favorable. During a clear, sunny day, tubers were exposed from 10:00 a.m. to 4:00 p.m. for a six-hour daily total, and kept until the next suitable day in a dark, slightly cool storage (10° to 15° C.). Exposures were carried out on the following dates: October 14, 17, 18, 19, 21 and 22, 1952. When the exposure time for each sample was completed, the tubers were prepared in the usual way for solanine analysis.

Analytical results are presented in Table 7, and illustrated in Figure 4.

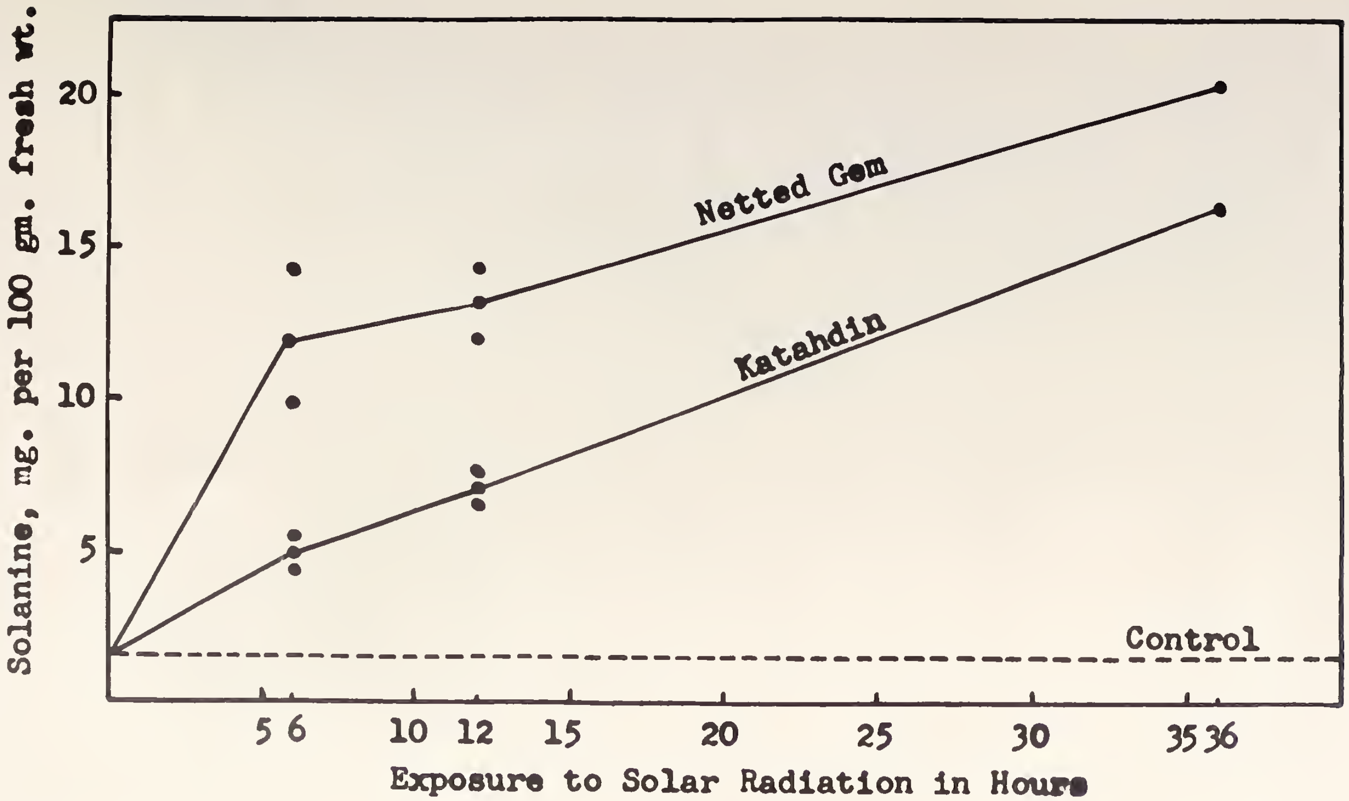


Fig. 4. Changes in solanine content of dormant tubers as affected by exposure to solar radiation at harvest, in Netted Gem and Katahdin varieties.

Table 7. Average solanine content of two potato varieties after various exposures to solar radiation at harvest time.

Exposure	Variety			
	Netted Gem		Katahdin	
hours	mg. % solanine	% increase	mg. % solanine	% increase
0	1.69	-	1.46	-
6	12.00	710	4.95	339
12	13.18	780	7.10	486
36	20.38	1206	16.28	1115

As a result of the treatment, traces of greening (chlorophyll formation) were observed at 12-hour exposure, and quite intense coloration was present after the 36-hour exposure. Katahdin tubers have shown slightly more intense greening than tubers of Netted Gem variety, even though they did not reach as high a concentration of solanine.

This was the only treatment included in the field experiments that established a very definite effect, and it represents a very valuable clue in further study concerning solanine synthesis of potato tubers.

The solanine content of control tubers was at the same low level as in all previous field treatments.

The steep rise of solanine content, especially in Netted Gem samples, after only 6 hours of exposure is impressive and significant. In one of these samples, a striking increase to almost 10-fold value of control tuber solanine content of 14.23 mg. percent was reached. The effectiveness of solar radiation upon solanine content of freshly harvested potato tubers seems very important if we consider the percentage increase, which is far beyond the normal expected variability.

The 12 and 36-hour exposures were carried out on two and six different dates, respectively. It was suspected that temperature during the exposure is of considerable importance, and if so, it would assumedly also influence the final solanine content. The temperatures were usually low in the morning and the afternoon, and the maximal reading did not exceed 60° F. The minimal readings ranged from 35° to 45° F.

Thus it seems that exposure of tubers to sunlight in late fall, when temperatures are generally rather low, may induce an extremely rapid synthesis of the alkaloid. This may or may not continue in subsequent storage. Hutchinson advances the speculation of bitter flavor development being due to a higher-than-normal content at harvest time, which may under subsequent storage increase faster than in tubers having a low solanine content to start with (25).

Facts disclosed by this experiment need further confirmation and ought to be extended to other varieties. The

trend of Netted Gem variety to produce readily larger amounts of solanine than tubers of Katahdin potatoes is indisputable, and will find further confirmation in storage experiments reported later.

The fact that dormant tubers of both varieties are able to synthesize very rapidly large amounts of the glycoside is a convincing suggestion that dormancy might be the critical stage at which abnormal synthesis of solanine in tubers is induced.

General Conclusions from Results of the Field Experiments

(a) In all treatments applied to potato plants at the end of the growing season, the solanine concentrations were found to be below what is considered a normal alkaloidal content, the levels in Carter's Early Favorite tubers being slightly higher than in those of Netted Gem and Katahdin varieties.

(b) Plants originated from solanine-rich Netted Gem potato seed have shown no differences compared to solanine content of plants from normal seed. Only in one case did the solanine level reach 3 mg. percent, and in only one other case exceed the 5 mg. percent level after mechanical removal of vines.

(c) Thus, it seems safe to conclude that none of the treatments applied to plants affected the solanine content of tubers to any appreciable extent. The experimental results thus seem to support the view of other workers that solanine content of potato plants and tubers decreases towards the end of the vegetative development period. This fact is suggested to be a result of destructive metabolism of solanine (46) and other alkaloids (26, 27) at this time, and possibly to the presence of some natural block to the transportation of the alkaloid from rich aerial portions into the tubers (46).

(d) Very short exposures of dormant tubers at the time of harvest to solar radiation appear to induce exceptionally rapid synthesis of solanine, and a further increase under subsequent cold storage may occur. The low temperatures at the time of radiation seem also to have some effect - an observation that needs further confirmation.

II. Greenhouse Experiments

In the summer of 1952, a few plants of Netted Gem potato variety were grown under greenhouse conditions. At a convenient time, these plants were exposed to various treatments in attempts to study the influence of certain factors upon the solanine content of the tubers.

Multiple-eye seed pieces were planted on May 27 in 8-inch pots, using a soil/sand mixture (3:1) as a growing medium. Temperatures in the greenhouse varied from 65° to 85° F., largely dependent on outside weather conditions. In order to increase the number of factors to be studied, only three to four plants were used for separate treatments.

Treatments were carried out as follows:

1. Three control plants were grown for 85 and 100 days, tubers being harvested on August 20 and September 5.

2. Chemical vine-killing using 5% sodium nitrate was carried out on August 20, tubers being harvested on August 27, after 92 days of growth.

3. Mechanical damage of vines was carried out by crushing and rubbing the plant tops with brick shale abrasive at the same dates as Treatment 2.

4. Additional phosphorus feeding to the plants was made at rates of 2, 4 and 6 gms., respectively,

of KH_2PO_4 per pot between July 11 and 20, in ten equal daily portions for each rate. Tubers were collected after 92 days of plant growth.

5. Abnormally high solanine seed pieces (NG II used in field experiments) were also planted, tubers being collected after 82 and 100 days of growth.

6. Short exposures of plants to infra-red light during normal daylight periods, totalling 50 and 100 hours, respectively, were carried out intermittently from June 20 to September 2, 1952. Tuber harvest took place after 100 days of growth, on September 5. Details of spectral energy distribution of the infra-red source used are given later in Figure 6.

7. Short exposures of plants to ultra-violet light during normal daylight periods, totalling 60 and 120 hours, were carried out intermittently from July 9 to September 2, and the experiment concluded on September 5 after 100 days of growth. Details of spectral energy distribution are given later in Figure 9.

The growth conditions of the plants were found to be quite unsatisfactory. Confined to inadequate 8-inch pots, these potato plants produced abnormally small tubers, the

highest pot yield not exceeding 150 gms. The vines appeared normal, though lighter than those of plants under field conditions. Nevertheless, tubers were collected and analyzed for solanine content. Complete results of analyses, including tuber yields, are presented in Appendix I, and the average values for solanine are given in Table 8.

Table 8. Average solanine content and percentage increases over control tubers Netted Gem potatoes grown under greenhouse conditions.

Treatment	Aver. solanine mg. %	Increase over control %
1. Control plants	8.39	-
2. Chemical vine-killing	8.92	6.0
3. Mechanical damaged vines	8.44	0.6
4. Additional phosphorus	6.23	-25.7
5. Plants from high-solanine seed	11.61	38.4
6. Infra-red light, 50 hours	10.05	19.8
" " 100 "	12.92	55.2
7. Ultra-violet light, 60 hours	13.10	56.1
" " 120 "	13.47	60.6

Discussion of Results and Conclusions

Because of abnormal tuber development, the solanine concentrations are generally higher than those found under field conditions. This probably was due to the small size of tubers, and therefore any interpretation as to the effect of treatments has to be made very carefully, and may be open to question. Taking the average of three plants, chemical vine-killing and mechanical damage to plants, as shown in Table 8, did not seem to affect solanine content of tubers to any appreciable extent when compared to controls.

The depression of solanine content by additional phosphorus seems to be quite significant, since the resulting decrease was 25 percent. It suggests the importance of mineral nutrition balance, and does not confirm observations of some workers that phosphorus may be responsible for an increase in solanine concentration (34). The matter should receive more attention by growing potatoes in nutrient solution cultures, in which the effect of low or high levels of various nutrients could be examined.

Abnormal growth conditions seem to present important physiological changes which may increase solanine content, and which are clearly visible in the plants grown from high-solanine seed. Whereas under field conditions solanine content of tubers ranged from 1 to 5 mg. %, under unfavorable and abnormal greenhouse conditions (small pots and relatively warm nights), the

tubers were abnormally small and have shown a high of 11 mg. % of solanine.

Both irradiation treatments of plants show increased alkaloid formation. Although the concentrations did not reach the dangerous limit of 20 mg. %, these concentrations probably would result in considerable bitterness if subjected to organoleptic tests, as noted in Appendix I. The effect of infra-red irradiation seems rather gradual and accumulative, whereas ultra-violet light increased the solanine content more abruptly after 60 hours of exposure and did not change after that time. Nevertheless, because of abnormal tuber development and the relatively small number of samples analyzed, the solanine concentrations as found cannot be interpreted as a result of treatments, and represent only leads towards further investigation.

ditions and harvested from the same fields had just normal contents of the compound.

Hutchinson, in preliminary trials (1950), reported the likelihood of low temperatures being more effective than relatively high temperatures in producing bitterness of Netted Gem tubers (25). Unfortunately, these observations were based on organoleptic tests of the cooked produce, and not on accurate chemical analyses.

With regard to previously mentioned observations, a series of experimental storage conditions was explored so as to note possible correlations with solanine formation.

The behaviour of two potato varieties, Netted Gem and Katahdin, respectively, was investigated under four different storage conditions. Single samples of four Netted Gem and three Katahdin tubers were selected for uniformity and divided into two lots:

In (1) a total of 12 samples of each variety were stored under normal storage conditions at temperatures of 4° to 8° C.

In (2) the same number of samples were kept in a dry storage place at temperatures ranging from 12° to 15° C.

Six samples of each lot were kept in complete darkness, the other six being exposed to a single weak Mazda light (15 W.) in a large radiation box with all other light

III. Storage Experiments

Bitter potato samples of the 1951 season were sent to the Plant Science Department of the University of Alberta from the School of Agriculture, Vermilion, Alberta. These were received in early March, and were obtained from a storage in which a Mazda light of 100 Watts had been left on continuously for about three days. Chemical analysis of these samples disclosed the following solanine concentrations (in mg. percent):

Netted Gem	23.46 to 25.20
Carter's Early Favorite	22.95 to 24.15
Canus	4.95 to 6.74
Katahdin	1.36 to 2.39

Other samples of bitter potatoes were obtained in early spring from a St. Albert producer, Mr. C. J. Robinson, who kept the potatoes in a small, damp and cool cellar with little aeration. These have shown interesting results noted below in mg. percent of solanine:

White Rose	5.01
Netted Gem	47.62

The foregoing analytical data underline the peculiar ability of Netted Gem and Carter's Early Favorite potato varieties to synthesize unusually large amounts of solanine, while the other varieties stored under identical con-

excluded. The light energy reaching these tubers from a 30 in. distance was below the sensitivity of a General Electric radiation meter (0.05 g.-cal./sq.cm./min.) and possibly did not amount to more than 0.005 g.-cal./sq.cm./min.

The experiment was begun on November 1, 1952, and samples for solanine analysis were taken at intervals of one week until December 13, when the experiment was concluded, giving a total of six weekly analyses for each storage condition.

Differences in fresh weight at the beginning and the end of each weekly interval were recorded, representing the total loss of weight due to respiration and transpiration. These values were required in order to recalculate the actual solanine content as analyzed in terms of 100 grams of original untreated tissue. Temperature fluctuations were also followed closely.

In summary, the variables studied were: two varieties, two temperatures, two light intensities, and six weekly intervals at which single samples from each individual treatment were collected. Analytical samples were halves of longitudinally-cut tubers, the remaining halves being discarded. These samples were prepared for analysis and quick-frozen as described earlier.

Complete results of analyses and percentage of weight loss are presented in Appendix II, and a summary of

solanine estimates is given in Table 9 and graphically illustrated for the Netted Gem variety in Figure 5. A total of 48 samples were analyzed in duplicates for solanine content.

Tubers exposed continuously to weak light developed traces of greening due to chlorophyll formation after one week, while distinct greening of much increased intensity was noticeable towards the end of the trial. The amount of greening was slightly less intense in the Netted Gem tubers than in the Katahdin variety.

Discussion of Results and Conclusions

The definite trend and ability of Netted Gem potatoes to synthesize under identical conditions larger amounts of solanine than tubers of Katahdin is obvious from the data in Table 9. As a matter of fact, conditions which in this instance induced additional synthesis of solanine in the former variety did not have nearly so appreciable an effect on the latter one. In one case only did the Katahdin solanine reach 7 mg. % of fresh weight, which was only slightly in excess of the normal solanine content for this variety of 5.5 mg. % as given by Wolf and Duggar (46).

Table 9. Total solanine content of two potato varieties as affected by various storage conditions. (All estimates recalculated to mg. percent of untreated tissue.)

Storage time in weeks	Cold, humid storage at 4° to 8° C.		Dry, warm storage at 12° to 15° C.	
	Dark	Light	Dark	Light
		Netted Gem variety		
1	7.93	19.83	7.50	7.20
2	5.63	19.01	7.96	5.04
3	11.30	18.51	3.52	3.20
4	13.75	17.38	5.78	7.26
5	11.09	16.34	4.01	3.65
6	15.42	23.50	8.72	6.98
		Katahdin variety		
1	2.65	2.73	4.27	2.57
2	1.46	3.20	5.64	3.16
3	1.76	4.64	3.07	1.66
4	2.55	4.32	3.48	1.72
5	2.14	2.76	3.27	1.97
6	3.11	7.01	6.06	5.02

1. Discussion of the Effect on Netted Gem

At dry storage with the higher temperatures, the solanine content apparently is not affected to any appreciable

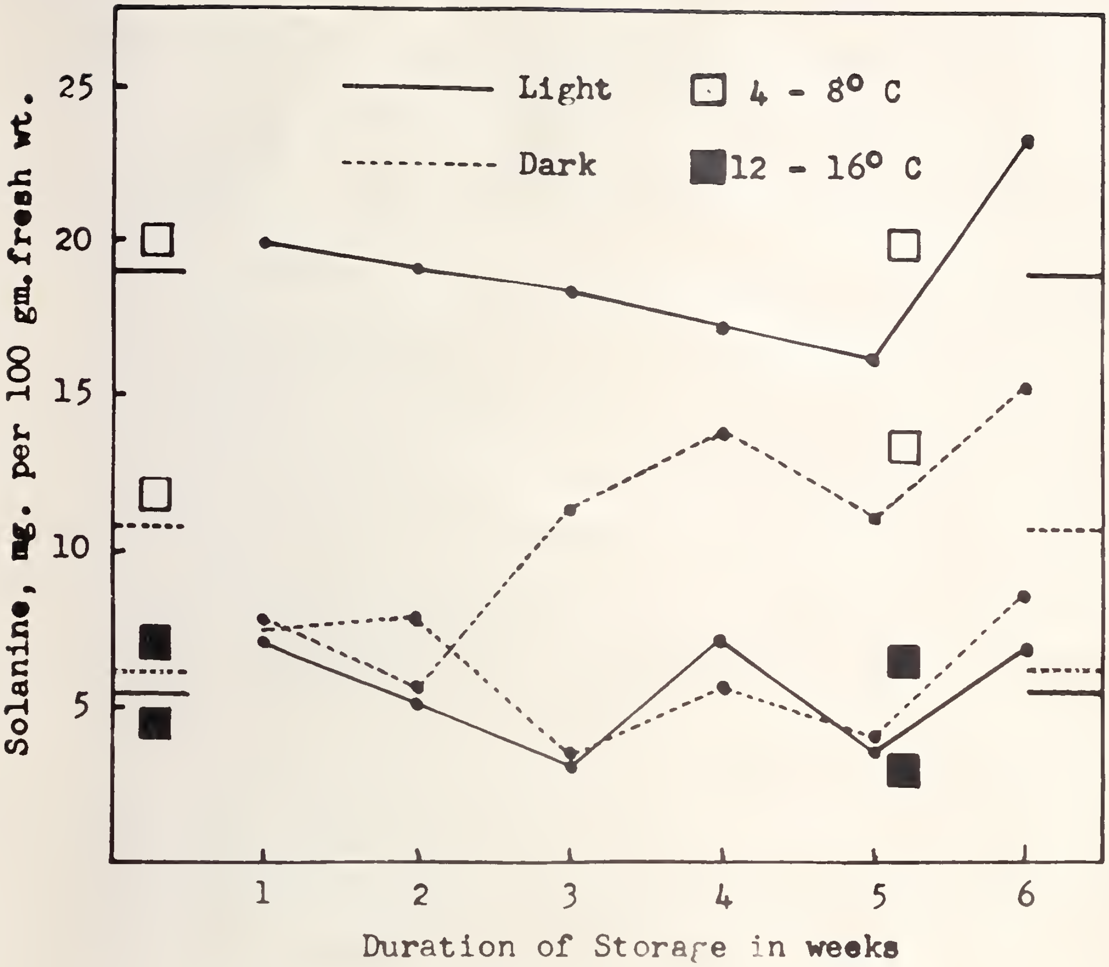


Fig. 5. Changes in solanine content of Netted Gem tubers as affected by various storage conditions during a 6-week period; average level of 6-week determinations indicated on the sides.

(Experiment carried out from November 1 to December 13, 1952.)

extent. The maximal values of 8.72 mg. % in dark, and 7.26 mg. % in continuous light, being only slightly higher than normal (4.7 mg. % according to Wolf and Duggar (46)). This amount probably could not be detected by organoleptic test.

On the other hand, under humid cool conditions at 4° to 8° C., whether in dark or in light, striking and rapid increases in solanine content are produced. This phenomenon is illustrated graphically in Figure 5. The marked effect is especially obvious if we take into consideration the averages of six determinations (disregarding duration of the storage) as indicated at the side of the graph.

The increase under continuous illumination is initiated much more quickly than that noted in the tubers in the dark, the latter being more gradual, and remaining well below the former after six weeks of storage. All illuminated tuber samples, and the four and six-week samples kept in the dark, are considered as "very bitter" because of dangerously high solanine content. The six-week sample exceeded the safety limit for food purposes of 20 mg. % solanine as determined by Bomer and Mattis (7).

If we disregard the storage duration and average all six determinations in this variety, the following values are obtained:

Humid storage at 4° to 8° C. -	Dark	10.86 mg. %
	Light	19.09 "

Dry storage at 12° to 15° C. -	Dark	6.26 mg. %
	Light	5.86 "

These summarized analytical data illustrate clearly the degree to which solanine content is affected under certain experimental storage conditions in Netted Gem potatoes. Thus it is not surprising, if the history of a bitter potato sample is traced, that cold and damp storage, sometimes with subdued light and possibly insufficient aeration, are coincident with the development of bitter and acrid flavor of tubers.

As this experiment was carried out shortly after the harvest of tubers, which were then at the beginning of their rest period, and as similar observations were made wherein tubers were exposed to solar radiation, it is concluded that dormancy is the critical stage at which (under favorable conditions in some varieties at least) high levels of solanine content can be reached most readily.

In both cases, the most rapid synthesis took place under illuminated storage at low temperatures. On the other hand, high temperatures and dry storage conditions appear to encourage a retarding effect upon solanine synthesis of this variety, and there is a possibility that the latter condition may bring about actual decreases in solanine content.

With regard to light and temperature, an inverse relationship seems to be indicated by the analytical data. The effectiveness of light on solanine synthesis seems to in-

crease with a decrease of temperature. The low temperature appears, however, to be a more important factor than light itself, as during low temperature storage a rise of solanine content in the tubers is noted whether or not they are illuminated.

The low temperature factor seems to point toward a disturbance or even an activation of an internal metabolic process, possibly involving some enzymatic action of unknown nature.

2. Discussion of the Effect on Katahdin

In the tubers of Katahdin, the results are not so clear-cut. Continuous illumination at low storage temperatures produced slightly more solanine than at higher temperatures, but when the tubers are kept in the dark this relationship seems to be reversed.

If treatment duration is disregarded, the average values of the six determinations may be compared as follows:

Humid storage at 4° to 8° C. -	Dark	2.28	mg. %
	Light	4.11	"
Dry storage at 12° to 15° C. -	Dark	4.30	"
	Light	2.68	"

If we consider the normal solanine content of this variety as reported by Wolf and Duggar (46) to be 5.5 mg. percent, and the highest determination of 7.01 mg. % as reached

under illumination at low temperatures, the data are not as consistent as are those for the Netted Gem potatoes. In spite of intense chlorophyll formation under the longest periods of illumination, it appears that this potato variety is less sensitive and is not predisposed to high solanine synthesis under the experimental conditions imposed. This may be due to the lack of some factor in Katahdin, which is responsible for excessive synthesis in Netted Gem tubers. The reverse may also be true; i.e., that a factor may be present in Katahdin and absent in Netted Gem to produce the same effect.

The chlorophyll formation in smooth-skinned Katahdin tubers was more intense than in the russeted Netted Gem potatoes. In the former variety, traces of greening were discernible after a few days of illumination. This is an indication that chlorophyll formation is not necessarily correlated with solanine synthesis, which bears out the work of Conner (12).

General Conclusions and Summary of Storage Experiment

(a) Solanine content of Netted Gem potatoes is considerably higher than in Katahdin tubers, as investigated under various storage conditions.

(b) Storage conditions, especially low temperatures, do affect to a marked degree the solanine content of Netted Gem, whereas the Katahdin tubers under the same conditions do not show a significant response.

(c) In Netted Gem tubers, temperatures of 12° to 15° C., whether continuously illuminated or kept in darkness, appear to have a rather retarding effect upon solanine synthesis. On the other hand, there is a definite and significant increase of solanine concentrations at low temperatures of 4° to 8° C. A relatively gradual rise is noted when tubers are kept in the dark, and a pronounced sudden increase to dangerously high levels of solanine takes place under continuous illumination even from a weak light source.

(d) The primary factor which is actually responsible for the disposition of this and some other varieties to produce large amounts of solanine rapidly, while others do not, remains as yet undetected. More fundamental research, to include a study of the amount and speed of solanine formation in more varieties, is needed before any definite conclusions can be drawn.

(e) The differences in humidity were also indicated; however, their importance cannot be stressed to any extent as no control work was carried out with regard to this factor. It may well be that relative humidity has contributed to the observed temperature effects.

IV. Irradiations of Netted Gem Potato Tubers

As various sources of light energy, including solar radiation, artificial light and ultra-violet light, have been repeatedly reported to influence the solanine content of potatoes, a series of experiments were designed to determine whether two specific components of radiant energy just above and below the visible spectrum would have a marked influence on the synthesis of the glycoalkaloid.

Continuous irradiations with ultra-violet and infra-red light sources of active and dormant Netted Gem potatoes were carried out and were correlated with the solanine content subsequently determined at the end of various exposure durations.

In the first experiment, physiologically active tubers of the previous season's crop, after 8½ to 9½ months of storage life, were used after the removal of sprouts. The sprouts were four to six inches in length. The infra-red radiation was applied between June 20 and July 6, and the exposures to ultra-violet light were carried out from July 6 to August 5, during the summer of 1952.

In late autumn, infra-red radiation was applied to freshly harvested dormant tubers for various time intervals from November 1 to November 22. In the concluding trial of this series, tubers stored for 3½ months (presumably just on

the break of the resting phase) were exposed to ultra-violet radiations between February 16 and March 6, 1953.

Considerable loss of fresh tuber weight was encountered, and was considered to be due to internal metabolic changes. These were recorded in order to enable recalculation of solanine content as analyzed in treated tubers to mg. % of tuber weight prior to the experiments.

A special cooling system had to be devised as a great deal of heat is given off by infra-red lamps. This heat raised the temperature at tuber surface to a level as high as 40° C. even when the light source was assembled at 53 inches from the tubers.

A wooden box 12x8x6 in. was prepared, around the sides and the top of which a glass-tubing coil was fitted. The box was inserted in a large earthenware tank filled with cold water, and cooling water was continuously run through the glass coil. At 3 to 5-hour intervals, a supply of ice-cubes was added to the larger tank. Thus the temperature of the cooling water was kept down to 12° - 16° C. A glass plate was placed over the tank so that it was in touch with the cool water of the large tank and also with the glass coil assembled over the top of the inner box.

Potatoes were placed onto this glass plate and further cooling was provided by a continuously running electrical fan. Thus a temperature of 22° to 26° C. was maintained on

the exposed tuber surface in the summer experiment, while when infra-red radiations were repeated in late fall, the temperatures were ranging from 18° to 22° C. due to the influence of outside freezing temperatures.

A photograph of the irradiation equipment is attached at the end of Appendix III.

1. Infra-red Irradiation

Infra-red light, according to Conner's report, had no particular effectiveness on solanine synthesis in tubers of Bliss Triumph potato variety (12).

His conclusions may be considered somewhat fallacious because of the fact that he did not pay close attention to the considerable amount of radiant energy from beyond the limits of the visible spectrum, which was transmitted by some of the Corning glass-filters employed. Conner concluded from his data that where the transmission of infra-red light was relatively intense an increase of solanine content resulted, but this was attributed to other components of the spectrum rather than to infra-red light.

It was felt worthwhile to investigate the behaviour of Netted Gem tubers on irradiations with a source radiating mostly infra-red light. This is thought to be a carrier of the fairly large amount of energy.

A pair of 250 W. R-40 General Electric or Fisher infra-red heat lamps were employed for this purpose. The distribution of relative energy as illustrated in Figure 6 indicates that very little of visible light and none of the ultra-violet zone is given off by this source. The total energy as measured at the surface of tubers by means of a General Electric radiation meter, Model 8 DW 60 Y 2 (at a distance of 50 inches) amounted to 0.32 g./cal./sq. cm./min., which is equal to 0.144 Watts/sq. in.

The earlier described cooling apparatus was necessary to lower the temperature at the tuber surface. The maximum intensity of the lamps used lies in the zone of 13,000 Ångstrom units, which is equal to 1.3 micron wave-length.

(a) Infra-red irradiation of active tubers after 8½ months of storage life

In early June of 1952, uniform Netted Gem tubers were selected for irradiation trials. Sprouts had developed to four to six inches, and these were removed. The tubers had been stored for eight and one-half months at 2° to 7° C.

As control samples, 4 tubers were analyzed immediately and another four were kept during irradiation exposures in the same room in darkness for 16 days (longest exposure). Temperatures at the surface of exposed tubers could not be held at less than 22° to 26° C., in spite of the cooling system used. The tubers were placed at a 50-inch distance from the lights.

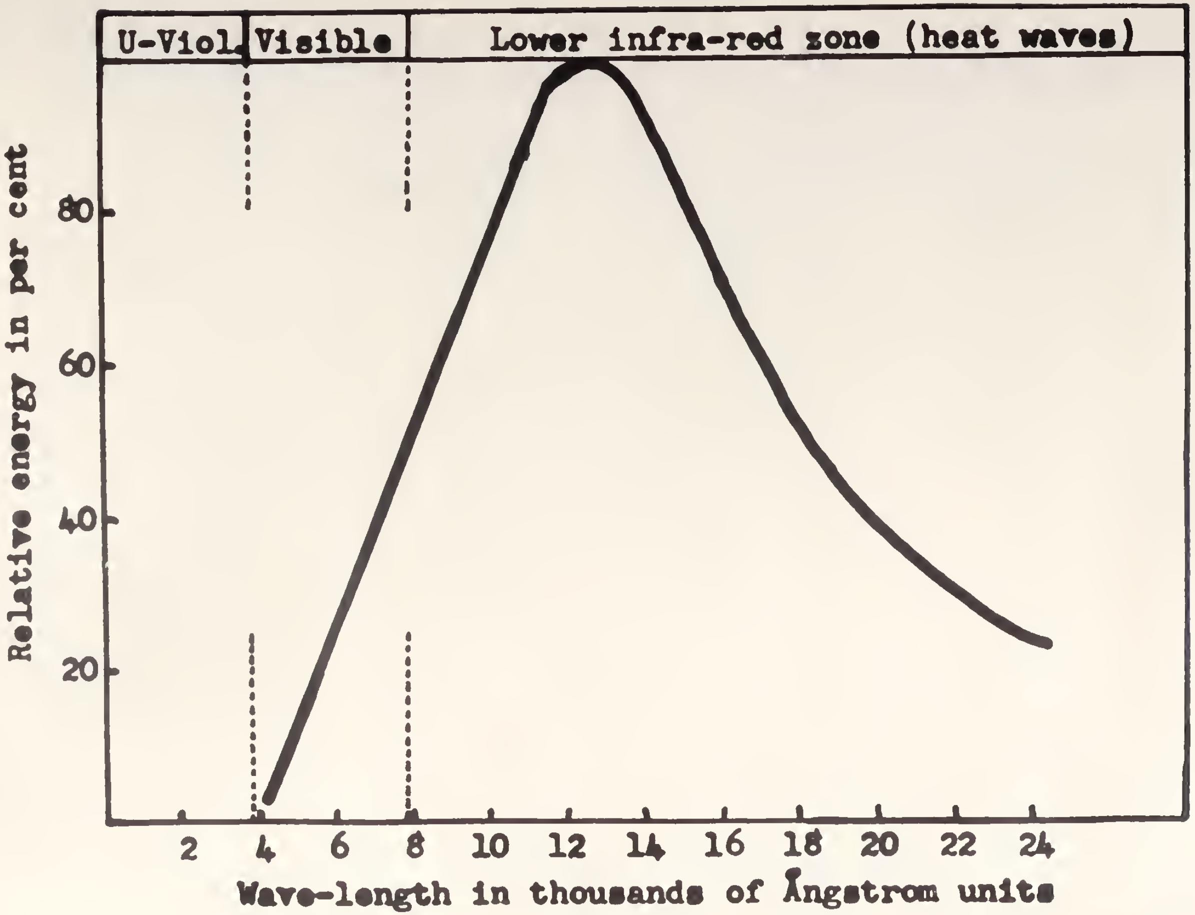


Fig. 6. Curve of spectral energy as radiated from a 250 W. infra-red heat lamp (R-40) with major zones of radiant energy spectrum indicated above.
(Data by courtesy of General Electric Co.)

The temperature difficulty can be appreciated when it is recorded that at one inch from the light bulb the recorded temperature was 78° to 80° C.!

The loss of weight of exposed tubers was recorded, and complete data for this trial are presented in Appendix III. The determinations of duplicate solanine analyses are given as means in Table 10.

Table 10. Mean readings of solanine content in Netted Gem tubers as affected by various intervals of continuous infra-red irradiation at 22° to 24° C., June, 1952.

Days of exposure	Total solanine mg. %	Sprouts gms.	Percent increase of solanine over dark stored control
0 (control, cold)	13.06	-	48
16 (control, dark)	8.85	3.5*	0
2	12.71	-	44
4	9.05	-	2
8	25.20	-	185
12	12.01	1.1	36
16	16.22	2.8	83

* This sample was kept in dark for 16 days at 22-24° C., hence the sprout development during that period.

As a result of the treatment chlorophyll formation was noted, traces of greening being visible after 8 days of continuous radiation. Intense green color was obvious after 12 and 16 days of exposure. Continuous exposures were carried out for 2, 4, 8, 12 and 16 days on single tuber-samples. Tubers of each sample were exposed alternately on each side, exposures for each surface being exactly half of the total exposure duration.

Due to high temperatures, the loss of weight was considerable, rising from 1.18% after 2 days to 6.89% loss of fresh tuber weight at the end of 16 days' exposure. In the two longest treatments a few weak sprouts developed, but their weight compared to total weight of the sample was negligible.

Discussion of Results and Conclusions

A considerable difference in solanine content between control tuber lots in cold and warm storage with no irradiation was observed. The former showed a relatively high amount compared to the latter, an increase of 48 percent.

A similar correlation was observed in the same variety in the fall storage experiment, as described earlier. This trend supports the suggestion that Netted Gem tubers at low temperatures are more likely to develop high solanine than ones at high temperatures. This effect, however, may be due to variability of solanine content in active tubers after a long

storage history, the extent of which is obvious from the analyses of irradiated tubers. The solanine content present in these irradiated tubers, though showing wide variation, is generally well above the content of control tubers kept in the dark at the same temperatures. Figure 7 also illustrates this effect. The results do not indicate with certainty that the increases are due to the infra-red treatment alone; nevertheless, dangerously high levels found in samples of 8 and 16-day exposures indicate that some synthesis of the alkaloid must have taken place.

In spite of the fact that the solanine content of tubers exposed for 16 days is well above that of the cold-stored control sample, it may be that the variable results of the treatments were primarily due to a wide initial variation in solanine content of individual samples at the beginning. This has been shown to be true in the next experiments using dormant tuber material, which had a very uniform initial solanine level.

Otherwise, the results of the experiment tend to support the Bomer and Mattis idea that on exposure to daylight old potatoes do not change solanine content as readily as do young mature tubers (7). This effect is probably due to renewed metabolic activity in tubers, which is undoubtedly intense at such high temperatures as those developed during this experiment.

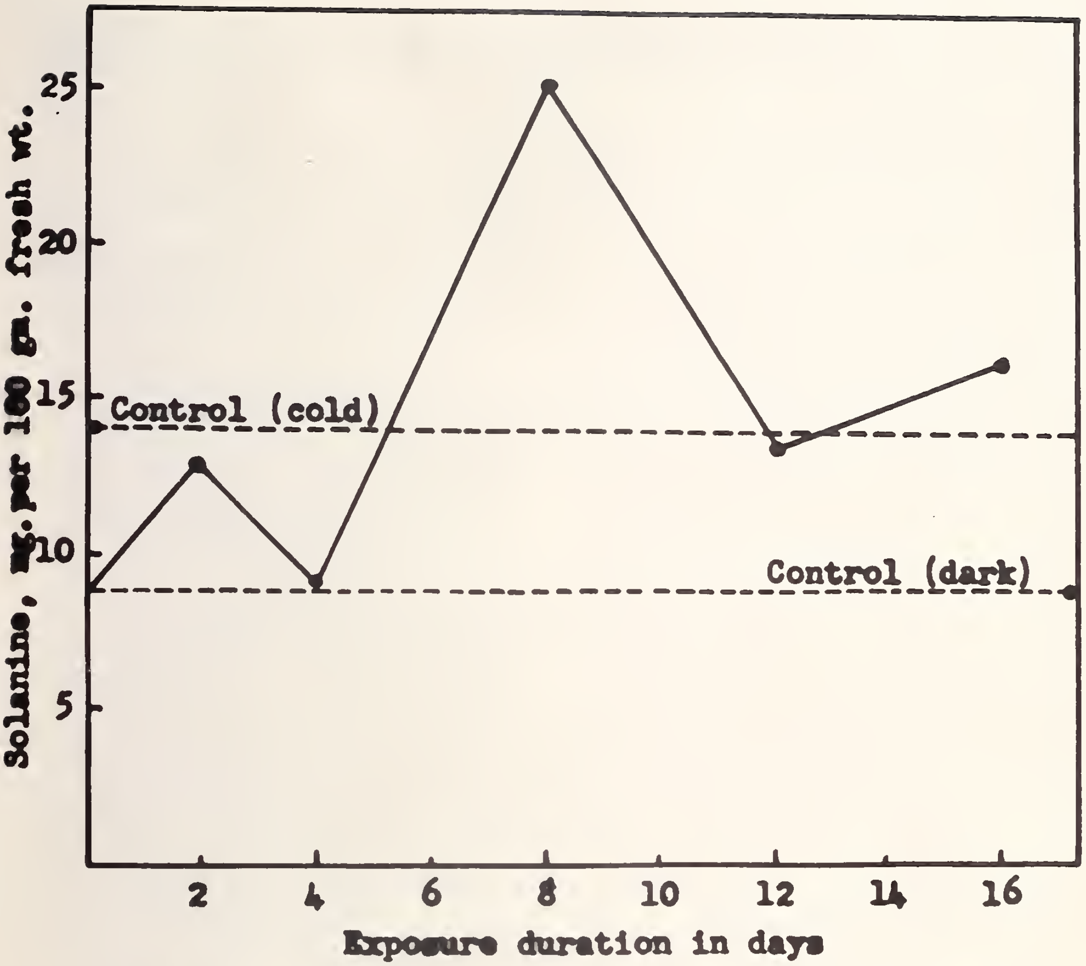


Fig. 7. Changes in solanine content of active Netted Gem tubers as affected by various exposures to infra-red light.

(b) Infra-red irradiations of dormant potatoes

Under the same conditions as in the previous experiment, freshly harvested Netted Gem tubers, after a short storage of 3½ weeks, were exposed in replicate samples to 4, 6, 8, 10 and 16 days of continuous illumination with infra-red light. Tubers of each sample were exposed alternately on each side, exposures for each surface being exactly one-half of the total exposure duration.

The experiment was divided into two phases: after the exposure was completed each sample of four tubers was divided into two halves, one of which was analyzed in duplicate immediately, the other half being stored under common cold storage conditions (4° to 8° C.) for two months. Triplicate analyses were carried out on the latter samples.

One sample was made up from four uniform medium-sized Netted Gem tubers. No sprouts were developed at any time during the experiment. Cold and dark stored single control samples were analyzed similarly to those in the previous experiment with physiologically active tubers.

Temperatures at the tuber surface were generally lower than in the corresponding summer experiment, being between 18° and 22° C. The percentage loss of fresh tuber weight did not exceed 4.12% in unstored tubers, but reached 9.65 percent in tubers stored for 2 months after the exposures to the infra-

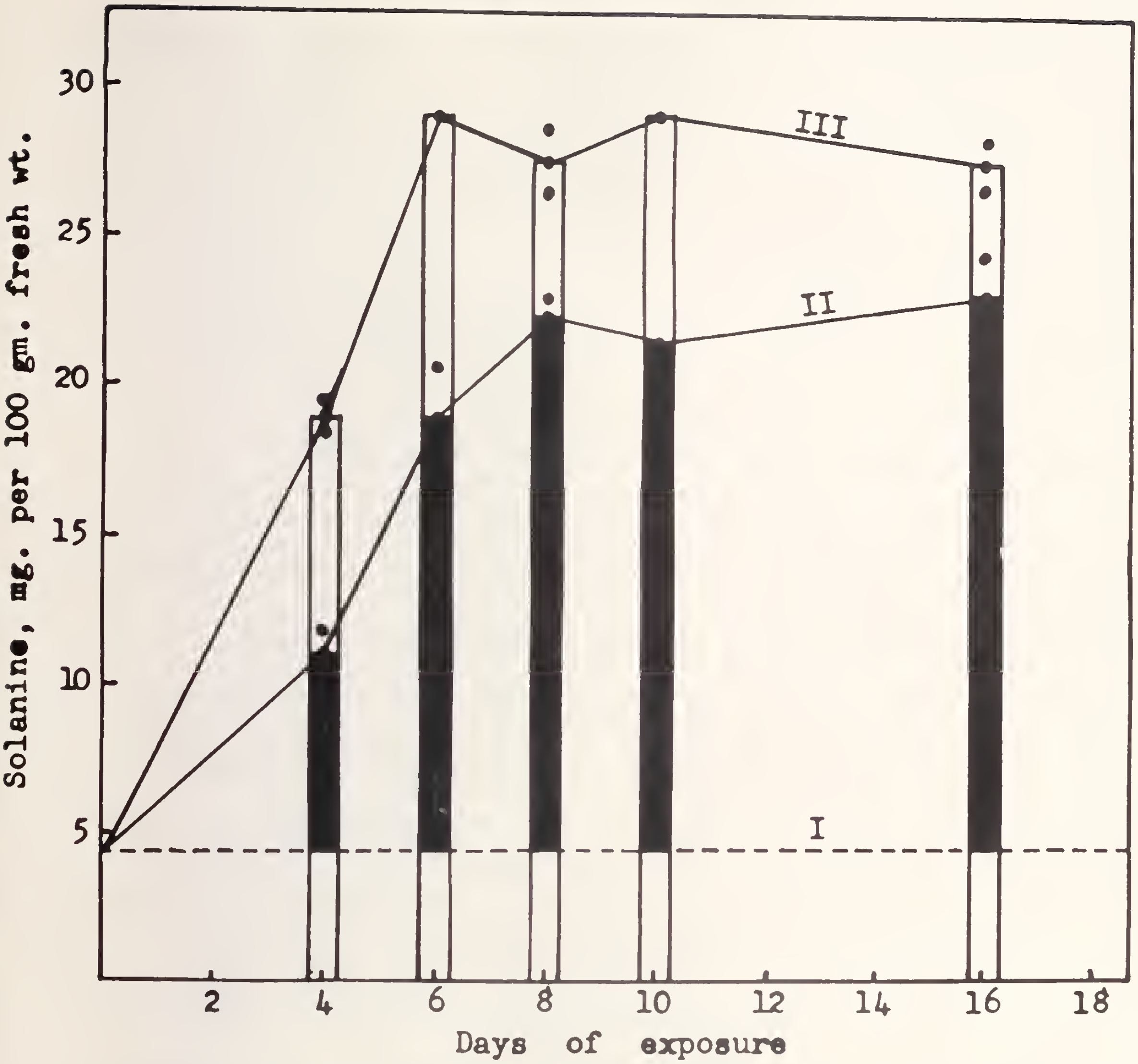
red light. The data pertaining to loss of weight are presented in Appendix IV-1.

Changes of solanine concentration as a result of irradiations are illustrated in Figure 8, and are summarized in Table 11.

Table 11. Solanine changes in dormant Netted Gems as affected by various exposures to infra-red light at 18° - 22° C. (November, 1952.)

Exposure days	Solanine mg. %	% Increase over unstored	% Total increase
0 - control	5.72	-	-
(a) Tubers analyzed immediately after treatment			
4	11.00	-	+92
6	18.88	-	+230
8	22.42	-	+292
10	21.54*	-	+277
16	23.17	-	+305
(b) Tubers analyzed 2 months after treatment			
4	19.17	+143	+235
6	28.87*	+175	+405
8	27.56	+89	+380
10	29.08*	+114	+391
16	27.50	+76	+381

* All figures represent the mean of duplicate analyses of two replicate tuber samples except those marked with an asterisk, in which case only one sample was analyzed.



I. Control, II. Treated and unstored, III. Treated and stored 2 months.

Fig. 8. Changes in solanine content of dormant Netted Gem tubers as affected by various exposures to infra-red light.

Again chlorophyll formation was noted in all samples. Compared to the greening in the summer experiment, it was concluded that much faster chlorophyll synthesis took place in irradiated dormant tubers, an intense color being noted after only four days of exposure. The samples exposed for the longest time had a very deep green color, reaching almost 3 to 4 mm. into the cortical tissue.

A secondary pigment formation of anthocyanins took place in tubers which were stored for two months following the treatment. This new pigment was most obvious in the shortest exposure sample, overshading almost completely the original chlorophyll with an intense purplish color. However, the chlorophyll did not disappear and was apparently unaffected in the cortical zone of tubers. The intensity of purple color decreased as the duration of exposure (prior to storage) increased, and in tubers exposed for 16 days only irregularly distributed blotches of purple were discernible when the tuber skin was carefully scraped off. The formation of anthocyanins is not a new record, as such a development was noted by Lampitt *et al* in sprouts which developed under continuous raying of tubers with ultra-violet light (28). No previous report has indicated the formation of anthocyanin pigment in tuber skins, however.

As there was little difference between the cold (6.78 mg. %) and the dark (4.65 mg.%) stored control samples, an average (5.72 mg. %) is used in the following discussion.

Discussion of Results and Conclusions

Table 11 illustrates the very rapid and significant increase of solanine concentration in dormant tubers after exposure to infra-red light. The solanine content was nearly double after four days of exposure, and was four times larger in the longest 16-day exposure when compared to control samples.

As indicated in Figure 8, the rise of solanine concentration was very steep in exposures up to 6 - 8 days, and very little change in solanine content took place after that.

As shown in Figure 8, the curve of solanine increase resembles the well-known curve of a limiting factor in photosynthesis (33). The steep rise at the beginning and the levelling-off of the curve at longer exposures (i.e., less solanine increase in spite of the radiation in samples where initial content is already high) may indicate the interference of some other factors essential in the synthesis which had become limiting. The accumulation of endproducts of a metabolic process may be such a limiting factor.

The increase due to storage following the irradiation, as shown in Table 11 - Part (b), is considerable in samples which had less solanine to begin with, whereas samples which had the highest solanine concentration at the beginning of the storage treatment showed less increase. These data clearly indicate that some additional solanine formation took place in samples stored after the irradiations. This corresponds to

the influence noted in other trials reported herein for storage under cool and humid conditions.

None of the irradiated tubers could be considered palatable, because of the dangerously high levels of solanine, as determined by a few organoleptic tests of raw ground tissue. A very distinct bitterness was noted in the four-day exposure sample, while the six-day sample showed such a degree of bitterness that the taste of it was retained for eight to ten hours, and consequently no more such taste tests were attempted.

It is possible that as a result of infra-red irradiation at high temperatures the free base solanidine was produced first, as indicated by exceeding bitterness detected through organoleptic tests; under cold storage conditions following the irradiation, the free base combined with the sugars, released on hydrolytic degradation of starch at low temperatures, to form the glycoalkaloid solanine.

The manifold increase in solanine content, even at temperatures as high as 18° to 22° C., is striking and significant. The results imply that this increase is primarily due to the effectiveness of long wave-length radiation, as it was shown in the storage experiment that high temperatures have a rather retarding effect upon solanine synthesis, and may even cause a degradation of the product to result in an actual decrease.

The data seem to confirm again that the dormant period of tubers is the decisive critical stage for promotion

of solanine synthesis, if conditions are favorable, and particularly in varieties such as Netted Gem that seem predisposed to the development of "bitterness." The considerable variability of solanine in active tubers after a long storage history indicates that the resumption of metabolic processes, especially at the beginning of the sprouting, does affect the solanine level within each individual tuber to an undetermined and probably irregular degree.

As was suggested by the storage experiments, the low temperature factor, becoming of more obvious influence with continuous illumination, apparently affects some internal system, possibly of enzymic nature. Similar speculative conclusions may be advanced from the results of the irradiation experiments. In spite of the high temperature conditions, which normally would favor enzymic action, the internal system, maintaining normal alkaloid content at a certain low level, is possibly inactivated by high light intensities. A similar influence is attributed to the "solarization" effect upon photosynthesis (33). It is also possible that the relatively high amount of energy carried by the infra-red light encourages the synthesis of such a complex molecule as solanine, as it is well-known that photochemical energy is required for many vital processes within a plant organism (8, 33).

General Summary and Conclusions from Infra-red Irradiation Experiments

(a) Active potato tubers of Netted Gem variety, after eight and one-half months of storage life, displayed variable amounts of solanine after various exposures to infra-red radiation. The variability is probably due to initial fluctuations of solanine content. Another factor influencing solanine level at this stage is probably the resumed metabolic activity which is the basis for the beginning of new growth.

(b) Dormant tubers of Netted Gem variety, on exposure to infra-red light of maximum efficiency in the 13,000 Ångstrom zone, responded by a rapid and very significant increase of the alkaloid content, as shown by chemical analyses and limited organoleptic tests.

(c) Dormant tubers which were stored after being irradiated showed an additional increase in solanine content. However, it is not certain if this increase was a sequence of the treatment or caused by subsequent cold storage, which itself favors solanine synthesis.

(d) An underlying internal metabolic process, possibly of enzymic nature, is postulated, a system which maintains normal equilibrium between alkaloidal synthesis and degradation. This system is presumably affected by low temperatures and by high light intensities, resulting in striking increases in tuber content of total solanine.

(e) All evidence points to the fact that the true rest period is the critical stage in which high levels of solanine in potatoes can be most readily developed.

2. Irradiations of Tubers with Ultra-violet Light

Various sources have reported that ultra-violet light is capable of promoting solanine synthesis (12, 28, 30). Therefore, a series of experiments were carried out with the object of determining what effect such light might have upon tubers of Netted Gem, a variety which is known to produce high solanine levels more readily than most other sorts.

Experiments were carried out under the same conditions as in the previous trials involving artificially produced light, with the exception that less concern was caused by high temperatures, as ultra-violet light sources have a relatively low energy output.

A single General Electric mercury vapor lamp (EH - 4 or B EH - 4 of 100 W. strength, equipped with a special filter) was employed for these investigations. The distribution of relative energy from this source is illustrated in Figure 9. The radiated energy in the 3654Å band amounted to only 3.25 Watts, and therefore the light was assembled at a four-inch distance from the tuber surfaces. There was still some transmission of light energy, chiefly from the blue-green end of the spectrum.

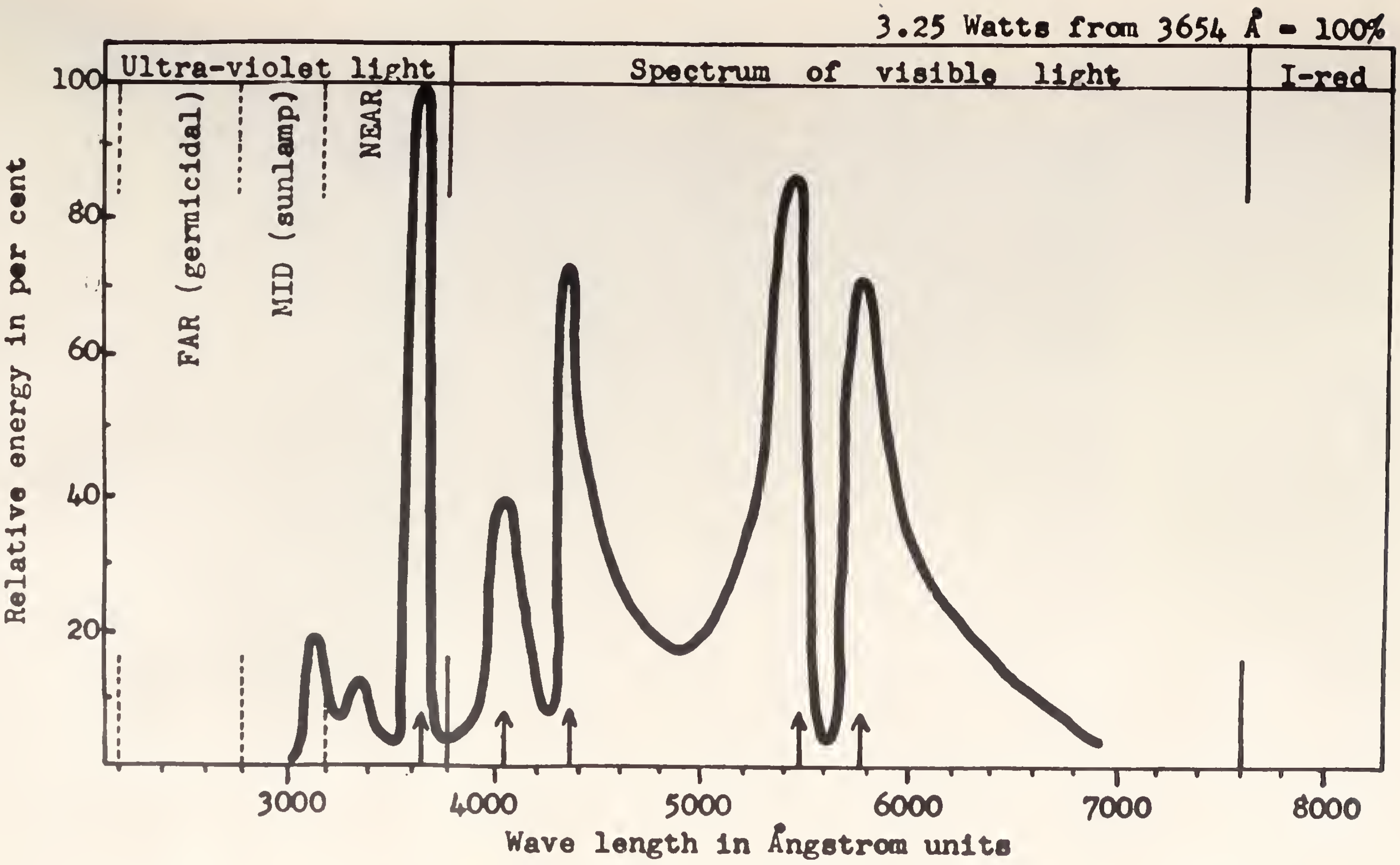


Fig. 9. Relative spectral energy distribution of B EH-4 General Electric ultra-violet lamp (100 W.). Principal spectral lines indicated by arrows are in 3654, 4047, 4358, 5461 and 5780 Å band.

(Data by courtesy of General Electric Co.)

The radiation meter as employed in previous trials did not show any response to this source of light, and the light energy at the tuber surface is thought to be well below the 0.05 g.-cal./sq.cm./min. sensitivity of the instrument.

(a) Ultra-violet irradiation of active Netted Gem tubers after 9½ months of storage life

Four uniform Netted Gem tubers per sample were selected, and the sprouts of 4 to 6 in. length formed in 9½ months of storage (at about 40° - 45° F.) were removed.

Control samples were treated in a manner similar to those in previous irradiation experiments.

The percentage loss of fresh weight due to respiration and evaporation was recorded for each individual exposure treatment. The data are reviewed in Appendix III.

Temperatures at the exposed tuber surface were about 18° to 22° C., kept continuously at this level by water circulating in the cooling system, as shown at the end of Appendix III.

The exposures ranged from four to twenty days of continuous raying of single tuber samples, and the exposed surface of tubers was alternated at short intervals.

No chlorophyll formation was noted in this experiment, nor was there any anthocyanin formation in developing sprouts such as was reported by Lampitt _et al_ (28).

The results of solanine analyses at the end of each exposure are summarized in Table 12, and illustrated in Figure 10.

Table 12. Solanine content in Netted Gem tubers as affected by various intervals of continuous exposures to ultra-violet light at 18° to 22° C. (July/August 1952.)

Days of exposure	Total solanine mg. %	Sprouts gms.	Increase over control in %
0 (control, cold)	13.06	-	-
20 (control, dark)*	12.36	28.5	-
4	4.91	-	-60
8	12.44	-	+0.6
13	15.62	1.8	+26
16	8.24	3.7	-33
20	5.20	4.7	-58

* Control sample from cold storage (4-8° C.) was analyzed prior to the experiment, and the other control sample was kept at a temperature of 18° to 22° C. in the dark for 20 days.

Discussion of Results and Conclusions

The analytical data of this trial again show considerable variation of the solanine content in physiologically active tubers under the imposed experimental conditions. The implications of the results suggest a decrease of solanine con-

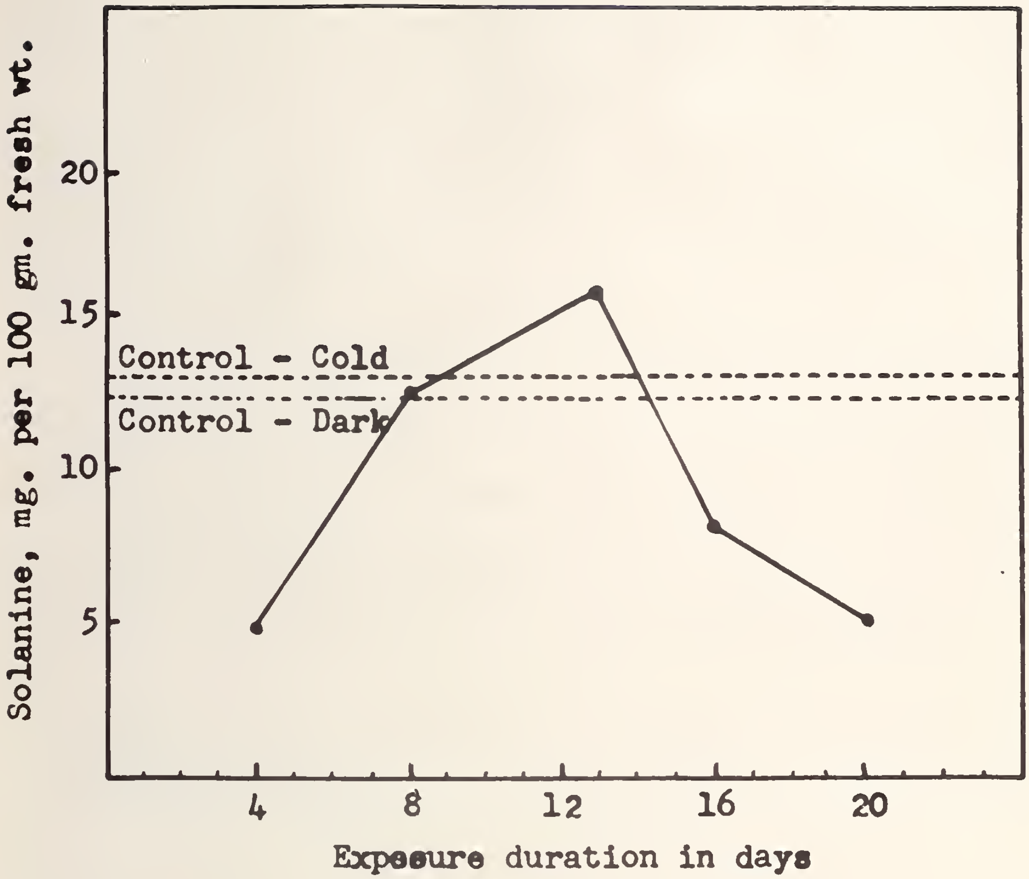


Fig. 10. Solanine content of active Netted Gem tubers after 9 months of storage as affected by ultra-violet light of maximum efficiency in 3654 A band.

tent on exposure to ultra-violet light. From the illustration in Figure 10, both control levels appear to be quite high and generally above those of irradiated tubers. However, having regard for the inconsistent solanine levels, which are similar to those in the first experiment with active tubers, no substantial conclusions can be drawn.

Because metabolic activity had been resumed, and perhaps also because a considerable number of sprouts had to be removed prior to the treatments, each individual potato probably had a different solanine level to begin with. This would, of course, lead to very inconsistent results.

(b) Irradiations of unsprouted potatoes after 3½ months of storage life

Due to the late shipment of replacement ultra-violet lamps, it was impossible to investigate the effect of ultra-violet rays upon late October-harvested tubers until mid-February. At this time, the rest period would be completed, even though no visible signs of sprouting could be observed.

Samples of four uniform tubers were used in this experiment, which was carried out under three different conditions, as follows:

(i) Irradiations under warm, dry storage conditions at 14° to 18° C., and under slightly humid, cold storage at 7° to 10° C. Each side of the tubers was alternately exposed to ultra-violet light for intervals of 4, 6, 8 and 10 days.

(ii) In another short experiment, tubers were exposed for 4, 6 and 8 days, but they were not turned to expose both sides. After irradiation tubers were halved and each half was analyzed separately. Temperatures during irradiation ranged in this case from 14° to 18° C.

Because the loss of fresh tuber weight during exposure times did not exceed much over one percent, no correction for this loss was taken into consideration, and the analytical data represent the amount of solanine present in treated tissue.

No chlorophyll or other pigment formation in any of the treatments was observed; however, signs of broken dormancy appeared in those exposures of six days and longer. The length of developing sprouts did not exceed one to two millimeters in the longest exposure.

Single samples were used under warm storage conditions, and duplicate samples of 4 and 8-day exposures were included in the cold storage experiment (i). Single samples, of four Netted Gem tubers each, were used in the latter experiment (ii).

Complete data of the experiments are presented in Appendix IV-2, and a summary of analytical results is given in Tables 13 and 14.

(i) The effect of ultra-violet light under two different storage conditions

The control and other samples were selected from a lot of potatoes which had on October 30 an average solanine content of 1.87 mg. percent. At the time of the experiment, three and one-half months later, a control sample from the same lot displayed 7.31 mg. percent of solanine. Another control sample kept in the dark for 10 days at temperatures of 14° to 18° C. had only 4.29 mg. percent of solanine. Such an apparent decrease in solanine content on transfer from low to higher temperatures has already been noted and discussed in some of the previous experiments (Tables 9 and 10).

From the results shown in Table 13 and Figure 11, a very peculiar behaviour of the solanine content in irradiated Netted Gem tubers was noted under the two conditions explored. While an apparent gradual increase of solanine concentration took place at higher temperatures, a rather remarkable reversal of this trend was observed under cooler conditions, and an abrupt decrease of the alkaloid is noted.

It is not easy to suggest an explanation of this reversal in the behaviour of solanine under the given conditions. It appears to be a new and as yet obscure factor of solanine synthesis. It may be that two enzymatic systems are involved, one of which shifts the equilibrium towards the synthesis of the alkaloid, while the other is supposedly antagonistic to the first one. This is, however, a speculation only,

and an extensive study would be required to substantiate any such possible explanation.

Table 13. Changes in solanine content of Netted Gem tubers as affected by continuous exposures to ultra-violet light under two different storage conditions. (February 1953.)

Days of exposure	Solanine mg. %	Percentage increase or decrease over	
		cold control	dark control
0 (control, cold)	7.31	-	-
10 (control, dark)	4.29	-	-
	(a) High temperature storage at 14° to 18° C.		
4	6.30	-	+47
6	10.63	-	+141
8	12.45	-	+190
10	13.82	-	+222
	(b) Cool storage at 7° to 10° C.		
4	7.44	+4	-
6	3.74	-49	-
8	3.33	-55	-

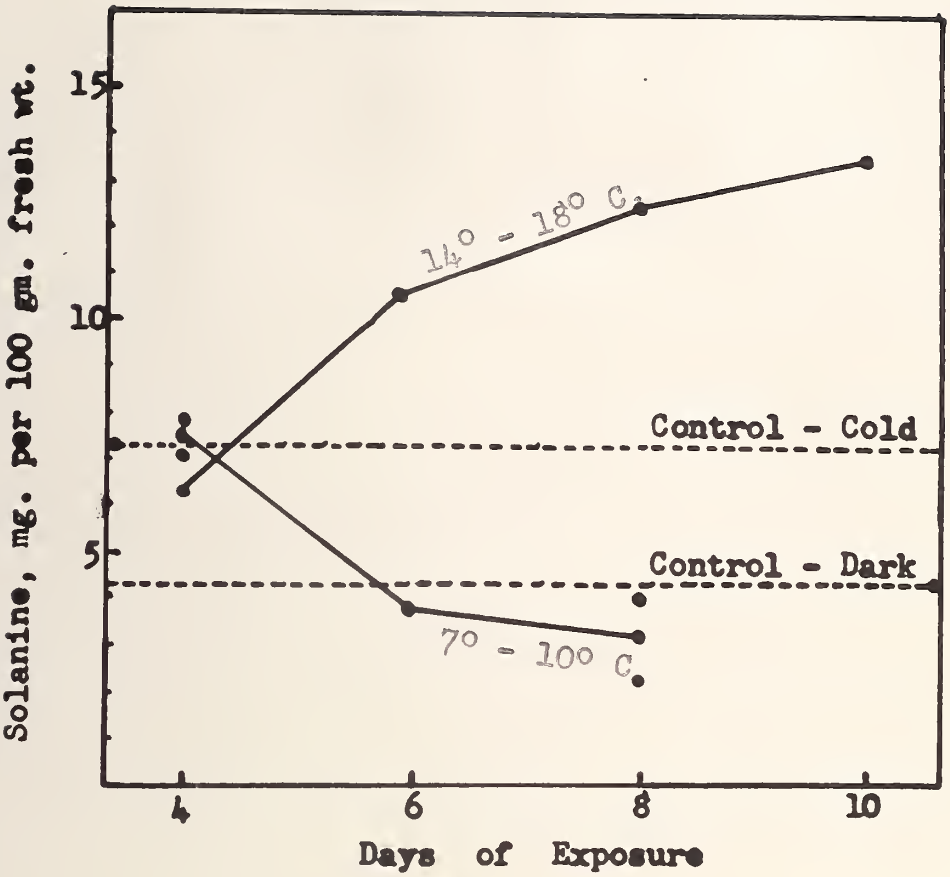


Fig. 11. Solanine content of Netted Gem potatoes exposed to ultra-violet light (with maximum efficiency in 3654 Å band) after 3½ months of storage life.

(ii) Differences in solanine content of exposed and unexposed halves of tubers as affected by exposure to ultra-violet light

When exposed and unexposed tuber halves were analyzed separately for solanine, the results being shown in Table 14, it was found that there is a general trend for the unexposed part of the tuber to produce slightly more solanine.

While 4 and 10-day exposures show a definite increase in solanine content, the latter to a dangerously high level of 18.38 mg. percent, there is an exceedingly low concentration found in the 6-day exposure. Because of a lack of completely undamaged tubers from the experimental material dug on October 30, 1952, slightly damaged tubers were used for the 6-day irradiation treatment. Whether or not the low solanine content in this sample is due to the injury, no possible explanation can be offered.

Table 14. Solanine content of Netted Gem tubers irradiated with ultra-violet light at 14° to 18° C. Exposed and unexposed tuber halves analyzed separately. February - March 1953.

Continuous exposure days	Tuber surface	Solanine content mg. %
4	(Exposed (Not exposed	10.90 9.72
6	(Exposed (Not exposed	2.60 4.10
10	(Exposed (Not exposed	18.38 22.85

Control sample kept in complete darkness at 14° to 18° C. = 4.21 mg. % of solanine.

In the 10-day exposure to ultra-violet light, the solanine produced considerably exceeded that of the same duration of exposure in the experiment reported in Table 13. This supports the idea that ultra-violet light at higher temperatures favors an increase of the alkaloid concentration. The recorded differences between exposed and unexposed tuber halves are so slight as to warrant no positive conclusions regarding the distribution within the tuber of ultra-violet ray effect.

General Conclusions of the Experiments with Ultra-violet Light

(a) Tubers that have just completed their rest period and that are irradiated with ultra-violet light at temperatures of 14° C. and higher show a gradual increase in solanine as the time of exposure increases.

(b) A reversal of this trend occurs if the exposure is carried out under cold-storage conditions at temperatures of 7° to 10° C., and for a period of more than four days.

(c) Analyses of unexposed and exposed tuber halves irradiated at relatively high temperatures indicate that the unexposed half tends to produce slightly more of the alkaloid. This may be a result of temperature differences between both surfaces.

(d) In active tubers after many months of storage, ultra-violet irradiation resulted in variable amounts of sola-

nine. The variation may have been due to resumption of metabolic activity, as indicated by the development of large sprouts. Presumably, at this stage, some internal factor or factors maintain the alkaloid-equilibrium at a certain level which varies widely between tubers.

(e) The ultra-violet light that was used in irradiation trials transmitted most intensity at the 3654Å band. As noted in Figure 9, however, other wave-lengths also were emitted, and these might have influenced the solanine concentration to some degree. Thus, any effect noted from ultra-violet radiation cannot be attributed exclusively to the wave-length of 3654Å.

GENERAL DISCUSSION AND SUMMARY

The experimental results have been discussed in some detail under each of the four environmental conditions that were created, and a few of the outstanding facts will be reviewed here.

(1) It was found that at the end of the growing season the solanine content was low throughout all analyzed samples, and that none of the treatments applied to growing plants, or to the plants near the close of the growing season, had any apparent effect. Hence the view of some workers that alkaloids are destructively metabolized towards the end of the growth period (26, 46) is supported by this work.

The situation becomes quite altered when dormant tuber material immediately after harvest is exposed to various treatments. Solar radiation at harvest time has an impressive influence in inducing rapid solanine synthesis in such a relatively short time as six hours. It is also thought that low temperatures at this time of the year also contribute to the effect. Thus it seems that the common practice of drying spread-out tubers harvested from wet soil may be one of the factors responsible for the development of bitter and acrid flavor in potatoes.

(2) From the greenhouse experiments, it can be concluded that abnormal growth conditions favor increased solanine

synthesis, which is counteracted to some extent by the addition of phosphorus in relatively gross dosages. The results from these experiments are not consistent enough, and merit further investigation with the use of nutrient-solution culture.

(3) The results of the storage experiments again emphasize the peculiar capacity of the Netted Gems to produce large amounts of solanine, if exposed to certain conditions that favor the synthesis of this compound. The Katahdin variety has shown little positive response to several treatment conditions, although from a solar radiation experiment it is obvious that this variety also is able to produce high concentrations of the alkaloid. It is possible that Katahdin has a more stable potential system which maintains the alkaloid equilibrium at a relatively low level, and therefore fewer specific factors are capable of upsetting its normal solanine level.

Humid and cold-storage conditions favor the alkaloidal synthesis in the Netted Gem as the storage period progresses, and the effect is impressively increased by a continuous exposure to weak light intensity which brings the concentration up to dangerous levels as high as 23.5 mg. %. Whether or not the effect of light is in correlation with photosynthesis, it is not known, although chlorophyll formation

may indicate such a relationship. The period during which solanine synthesis may take place most readily appears to be prior to the conclusion of the rest period, and certainly prior to resumption of active growth.

On the other hand, dry storage at high temperatures, at least above 10° to 12° C., definitely retards alkaloid production and maintains it at low levels. It was therefore postulated that the increase at low temperatures may be a result of the activity of a single factor; possibly a synthesizing system is inactivated by temperatures above 10° C. This system may be carrying out one of the final steps in alkaloid formation. The predisposition of Netted Gem may be due to a presence of higher levels of certain substances which are utilized as precursors of solanine. It is known that enzymic hydrolysis of starch favors increased formation of free sugars and sugar intermediates, as was shown in Table 1. There seems also to be some correlation of solanine with protein synthesis and degradation.

(4) The exposure of tubers to infra-red radiation has a definite and pronounced effect upon the synthesis of solanine at least in dormant tubers. A very high degree of bitterness was observed on organoleptic tests of ground raw tubers; it is possible that infra-red treatment favors the synthesis of the free alkaloidal base, as a further increase of total solanine content was observed following a subsequent

period of two months' cold storage. It was therefore suggested that the free solanidine would combine with the sugars released through enzymic action at low temperatures (8). Increases up to four times were noted in irradiated tubers over the control samples, and dangerous levels as high as 27.50 to 29.08 mg. % of fresh tuber weight were recorded.

It is suggested that further information is required as to the behaviour of irradiated tubers under subsequent dry storage at higher temperatures.

The experiments with active material, that previously was kept for over nine months in cold storage, have shown inconsistent results, probably to a large extent due to the between-tuber variability in solanine content, as influenced by active metabolic processes taking place. The tubers at a physiologically active stage probably are able to maintain the alkaloid at relatively low levels.

Chlorophyll formation on tubers was noted in samples after a few days' exposure, the greening being more intense in dormant than in active tubers.

(5) Ultra-violet light of maximum intensity in the 3654Å band of the spectrum exhibits strange influence on the alkaloid production. Metabolically active tuber material, after nine months of cold storage, shows a rather variable response upon

exposure to ultra-violet rays, though there appears to be some decreasing effect.

Tubers of only three and one-half months of storage life, no doubt just at the limit of their rest period, increase the concentration of solanine if rayed at temperatures of 15° to 18° C., whereas at lower temperatures of 7° to 10° C. a reverse response was found. The increase under the former conditions was more or less gradual, while under the latter the decrease was rather abrupt after exposure for six days.

No chlorophyll formation was observed in either case, indicating that ultra-violet light does not promote chlorophyll development.

(6) On the basis of experimental data and observations reviewed here, and from the literature reviewed earlier, the following attempt is made to develop a hypothesis concerning the synthesis of solanine in potatoes:

Based on recent achievements in the fields of genetics and plant biochemistry (4, 5, 6, 8, 26), enzymology (18, 42) and plant physiology (33), the presence of a primary functional system in the growing potato plant is postulated; this system controlled by genetic constitution maintains normal alkaloid levels in the plant, i.e. a rapid synthesis of solanine

at an early stage of growth and a natural degradation of the product towards the end of the vegetative period (26, 27). Such a system may be expected to be present in tubers as well as in vegetative above-ground plant parts.

The primary "regulating" system is, in certain cases, secondarily affected by conditions of the environment of the plant material, and the dormant phase seems to be the critical stage at which the regulating system (and thus the solanine content) is most easily and markedly affected.

From observations in storage and infra-red irradiation experiments, it seems probable that the postulated function is rather antagonistic to rapid solanine synthesis and/or to high levels of the compound; i.e., its function may involve or include metabolic destruction of the alkaloid when under favorable conditions the compound builds up to higher-than-normal concentrations. Inhibition of this system, such as may take place through low temperatures or high intensity of long wave-length light, may consequently result in accumulation of solanine. This primary system is possibly correlated with the "natural block" suggested by Wolf and Duggar (46), which prevents any translocation of solanine to the tubers at the end of the vegetative period.

The varietal differences, due to some hereditary character, are perhaps of minor importance since environment plays a very decisive role in abnormal solanine formations.

Nevertheless, the predisposition of certain varieties to produce high solanine levels more readily than other varieties is possibly due to some specific genetic character, as it is known in many instances that internal metabolic processes are under genic or enzymatic control (5, 6).

The ability of Netted Gem and Carter's Early Favorite tubers to produce high solanine levels more readily than other varieties, such as Canus, Katahdin and White Rose, mightbe explained on the basis of certain variability in the stability of secondary internal metabolic processes. The former varieties apparently possess compounds and precursors which are much more readily (and in a relatively small number of steps) transformed to solanidine; while the latter varieties do not have any abundance of solanine precursory compounds or metabolites, and therefore many and more complicated steps are necessary to bring about their final synthetic development toward the solanidine molecule.

Studies of methionine synthesis in Neurospora show that it is dependent on the presence of about seven different genes, each of which controls one particular step in the synthesis. If one of these steps is blocked by gene mutation (elimination of one of the particular genes involved), the organism is unable to accomplish the methionine synthesis, and consequently metabolites of the preceding steps of the synthesis are accumulated (23). Although methionine is a

relatively simple essential amino acid, similar processes may be involved in such a complex synthesis as that of solanidine and solanine. The lack of one particular gene or enzyme, functioning in or governing any one particular step, may account for the inability of some varieties to build up solanine rapidly. Another variety which possesses the necessary genic or enzymatic complement will have no difficulty in inducing rapid synthesis of solanine.

Conversely, it may be that an enzyme or enzymes (governed by certain genes) may be vital in the degradation of solanine and solanidine, and also in the speed of such a break-down. Therefore, the lack of such a genic complement may result in the withholding of solanine in the plant tissue; in some respects a reversal of the system outlined in the preceding paragraph.

The low temperature and high light intensity effect certainly suggest some enzymatic inhibition, which in most cases is dependent on the presence of certain genes (4, 5). The metabolic changes at very low temperatures, as in the increased respiration of tubers, or in the hydrolytic degradation of starch (8, 33), undoubtedly play an important role during the tuber storage life, particularly during the resting phase. Also, photosynthesis, as indicated by rapid chlorophyll synthesis in irradiated tubers, may be involved in the problem, and should receive more attention.

Bonner holds that alkaloids arise as secondary by-products irreversibly formed from accumulated essential metabolites (8). If this is true, metabolic changes, as suggested in the previous paragraph, will certainly be in favor of alkaloid formation, as the energy released by such processes must be utilized elsewhere.

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In spite of the fascinating progress which has been made in biochemistry, genetics and plant physiology in recent years, there still remains much to be elucidated concerning the fundamental metabolic changes in such complex living systems as occur in the infinitely variable members of the plant kingdom.

ACKNOWLEDGMENTS

The research work presented herein was carried out in the fields and laboratories of the Plant Science Department at the University of Alberta.

The financial aid was provided largely by a grant from the National Research Council of Canada, which was partly used for the purchase of additional equipment and instruments; and further, through a University of Alberta Research Scholarship, awarded to the writer. This assistance is much appreciated.

Sincere thanks are also expressed to Dr. R. J. Hilton, who stimulated the investigation in this field, and who directed the work throughout by valuable suggestions and able guidance.

Technical advice from Dr. E. H. Gowan, Department of Physics, University of Alberta, is also acknowledged. The writer also wishes to express his thanks to Mr. C. J. Robinson, St. Albert, Alberta, from whom seed tubers with high solanine content were received for experimentation.

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APPENDIX I

Estimates of total solanine in tubers of greenhouse grown Netted Gem potatoes exposed to various treatments

	Treatment		Days of growth	Wt. of tubers gm.	Total solanine mg/100 gm.
I	Control plants	1	85	72.1	7.80
		2	85	95.6	9.79
		3	100	83.2	7.57
II	Chemical vine killing	1	92	84.2	8.62
		2	92	101.6	9.98
		3	92	89.7	8.15
III	Mechanical damage of vines	1	92	108.1	5.36
		2	92	107.0	9.15
		3	92	94.4	10.82
IV	Additional phosphorus feeding (KH_2PO_4)	2 gm.	85	105.7	5.15
		4 gm.	100	122.8	7.38
		6 gm.	100	144.6	6.17
V	High-solanine seed planted	1	100	88.2	10.26
		2	100	117.1	11.06
		3	100	67.3	13.52
VI	Infra-red lamp irradiated plants	50 hr.	100	76.3	9.40
		50 hr.	100	69.7	10.70
		100 hr.	100	87.8	10.16
		100 hr.	100	93.9	15.67
VII	Ultra-violet lamp irradiated plants	60 hr.	100	55.9	13.60
		60 hr.	100	76.0	12.59
		120 hr.	100	83.2	14.77
		120 hr.	100	70.1	12.16

APPENDIX II

Estimates of total solanine content and percentage of weight loss in dormant Netted Gem and Katahdin potatoes as affected by various storage conditions during a 6-week period

1. Netted Gem potatoes

Storage, weeks	Percentage loss of fresh wt.		Mg. per cent solanine	
	Dark	Light	Dark	Light
	Humid, cool storage at 4 to 8°C.			
1	2.35	2.32	7.93	19.83
2	2.03	2.35	5.63	19.01
3	2.07	2.89	11.30	18.51
4	2.15	3.00	13.75	17.38
5	3.15	4.17	11.09	16.34
6	3.66	4.41	15.42	23.50
	Dry, warm storage at 12 to 15°C.			
1	2.99	3.29	7.59	7.20
2	3.01	3.97	7.96	5.04
3	3.47	4.96	3.52	3.20
4	3.94	5.23	5.78	7.26
5	4.62	6.03	4.01	3.65
6	5.33	6.26	8.72	6.98

Time of storage: November 1 to December 13, 1952.
Light source: 15 watt Mazda lamp, 30 in. from tubers.
Weight of 4-tuber sample: 500 to 700 gm.

APPENDIX II - (Continued)

2. Katahdin potatoes

Storage, weeks	Percentage loss of fresh wt.		Mg. per cent solanine	
	Dark	Light	Dark	Light
	Humid, cool storage at 4 to 8°C.			
1	3.60	3.62	2.65	2.73
2	3.20	3.61	1.46	3.20
3	3.32	4.00	1.76	4.64
4	4.75	4.26	2.55	4.32
5	4.70	5.66	2.14	2.76
6	5.16	6.24	3.11	7.01
	Dry, warm storage at 12 to 15°C.			
1	3.58	3.12	4.27	2.57
2	3.50	3.69	5.64	3.16
3	3.84	4.10	3.07	1.66
4	4.70	5.35	3.48	1.72
5	5.76	6.00	3.27	1.97
6	6.43	6.71	6.06	5.02

Time of storage: November 1 to December 13, 1952.
Light source: 15 watt Mazda lamp, 30 in. from tubers.
Weight of 3-tuber sample: 750 to 1,050 gm.

APPENDIX III

Solanine content and other data.

Active tubers of Netted Gem irradiated with infra-red and ultra-violet light

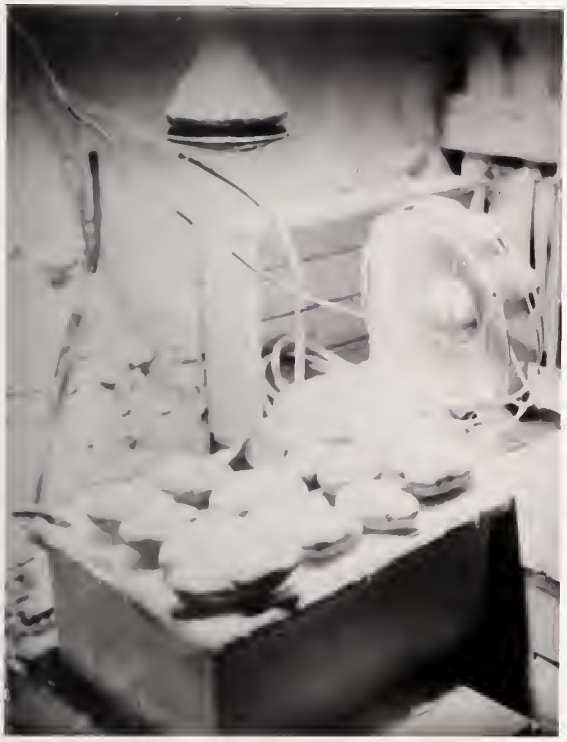
Exposure, days	Solanine, mg. %	% increase over dark control	Sprouts gm.	Loss of fresh wt., %
0 (Control, cold)	13.06	-	-	-
16 (Control, dark)*	8.85	-	3.5	6.73
20 (Control, dark)**	12.36	-	28.5	13.13
Infra-red irradiations				
2	12.71	+ 44	-	1.18
4	9.05	+ 2	-	1.79
8	25.20	+185	-	3.37
12	12.01	+ 36	1.1	4.31
16	16.22	+ 83	2.8	6.98
Ultra-violet irradiations				
4	4.91	- 60	-	2.13
8	12.44	+ 0.6	-	3.92
13	15.62	- 26	1.8	5.62
16	8.24	- 33	3.7	7.31
20	5.20	- 58	4.7	9.49

All solanine estimates are recalculated to mg. per cent of fresh weight at the beginning of individual treatments.

* Dark stored control sample of the infra-red treatment carried out during June 20 to July 6, 1952.

** Dark stored control sample of the ultra-violet treatment carried out during July 6 to August 5, 1952.

APPENDIX III - (Continued)



Photograph showing irradiation apparatus assembled in a dark room. In the foreground, the potato tubers are lying on a glass plate placed over a cooling water tank. An electric fan provides continuous air exchange. The ultra-violet lamp, shown above, is lowered to a position four inches above the tubers when these are being irradiated. The source of infra-red light is not included in the picture as it is 50 inches above the tubers.

APPENDIX IV

Complete data on Netted Gem dormant tuber irradiations with infra-red and ultra-violet light

1. Infra-red irradiations

	Unstored tubers			Stored tubers***		
Exposure days	Solanine mg. %	% increase	% loss of wt.	Solanine mg. %	% increase	% loss of wt.
0*	7.92 5.63					
16**	3.52 5.78		3.47 3.94			
4	11.73) 10.26)	92.31	0.34 0.45	18.88) 19.45)	235.14	3.15 2.19
6	17.13) 20.63)	230.07	1.18 2.52	28.87	405.07	5.91
8	23.94) 20.89)	291.78	2.41 3.01	26.52) 28.60)	379.89	5.38 7.23
10	21.54	276.57	3.81	29.08	390.91	9.65
16	24.57) 21.75)	304.89	4.12 3.87	28.40) 26.59)	380.59	8.74 8.48

Total percentage increase of solanine content over control sample are given as average of replicates except in 6-day exposure stored and 10-day exposure for both stored and unstored, which were carried out on single tuber-samples.

* Cold-stored control sample analyzed prior to the experiment.

** Control tuber sample stored in dark for 16 days at temperatures of 22 - 26°C.

*** Tubers stored for two months after the indicated exposure.

APPENDIX IV - (Continued)

2. Ultra-violet irradiations

A. Under two different storage conditions

Exposure, days	Percentage loss of fresh wt. 7-10°C.	Percentage loss of fresh wt. 14-18°C.	Mg. % of solanine 7-10°C.	Mg. % of solanine 14-18°C.
0 (control, cold)*			7.31	
10 (control, dark)**		0.91		4.29
4	0.75	1.16	7.44	6.30
6	0.79	1.14	3.74	10.63
8		1.31		12.45
10	0.98	1.77	3.33	13.82

B. Differences between exposed and unexposed surface of tubers

Exposure, days	Loss of wt. %	Mg. % of solanine Exposed	Mg. % of solanine Unexposed
4	0.56	10.90	9.72
6	0.72	2.60	4.10
10	1.65	18.38	22.85

* Cold-stored sample analyzed prior to the experiment.

** Control tuber sample stored in dark for 10 days at temperatures of 14 - 18°C.

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